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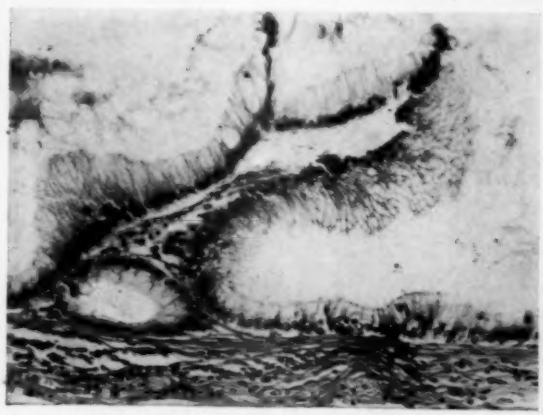

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A.M.A. Archives OF PATHOLOGY

Studies on Atopic Dermatitis

I. Connective Tissue Changes and Their Alteration Following Treatment with Corticotropin and Adrenocortical Hormones

BEN Z. RAPPAPORT, M.D., Chicago

In 1950 [1] we observed that during seasonal hay fever changes occur in the connective tissue of the nasal mucosa and that these alterations are reversed by corticotropin. Using the McManus-Hotchkiss * method for staining carbohydrate-protein compounds, it was observed that during the allergic state the ground substance of the nasal mucosa stains very lightly and that the basement membranes below the mucosa and about the blood vessels are thinned. Following treatment with corticotropin, the ground substance and basement membranes regained the staining characteristics of normal nasal mucosa. These observations led to the hypothesis that changes in connective tissue are important in the immediate just as in the delayed type of allergic reaction. Adrenocortical hormones, through their effect on connective tissue, tend to reverse the reaction in the former type of allergy. As it has been questioned whether the wheal type of allergic reaction, frequently demonstrable in atopic dermatitis, is related to the condition, it seemed relevant to determine if responses similar to those in nasal allergy could be observed in the connective tissue of atopic dermatitis before and after treatment with certain hormones.

Histologic examination of the skin of patients with atopic dermatitis was made at the period of active inflammation and following treatment with corticotropin and adrenocortical hormones. The marked changes in the ground substance and basement membranes present before treatment were reversed by the hormones used.

METHODS

The patients selected for this study had marked, active atopic dermatitis. It had been present since infancy or early childhood in all except one, in whom the dermatitis had been noted two years previously. There were 10 women, ranging in age from 26 to 40, and 5 men, from 21 to 26 years of age.

Two morphologically similar sites in an area of dermatitis within a radius of 2 cm. were selected for biopsy before and after treatment. Occasionally, corresponding sites on the flexural surfaces of each arm were found most similar and were therefore used. For anesthesia, procaine (2%) was injected around the selected site, with care to avoid infiltration into the biopsy area. Biopsy specimens were made with a 5 mm. punch before treatment, and again at the height of improvement, usually 10 to 14 days after the beginning of therapy. Tissues were also obtained from corresponding normal-appearing skin areas before and after treatment.

Immediately after removal, the skin specimens were frozen at —150 to —160 C in isopentane cooled by liquid nitrogen and then were dehydrated *in vacuo* at about —30 C, according to the method of Altmann-Gersh.[4] This method of tissue preparation produces minimal artifacts, avoids denaturization of proteins, and arrests activity of enzymes without destroying many of them.

Submitted for publication March 18, 1955.

This work was supported by a grant from the Asthmatic Children's Aid of Chicago.

From the Department of Medicine and the Allergy Unit, University of Illinois College of Medicine.

* References 2 and 3.

After dehydration, the tissues were prepared for histologic study by infiltration with paraffin (M.P. 52 to 56 C). Sections were stained for formed elements with hematoxylin and eosin, Giemsa, and toluidine blue, and for mucoproteins of the ground substance † with the McManus-Hotchkiss method.‡ Since the staining intensity of the ground substance and of the basement membranes is influenced by the thickness of the sections, care was taken to standardize this factor by the selection from each tissue of four to six sections cut at 4μ and a similar number at 8μ. All of these were mounted on the same slide. This permitted an estimate of the uniformity of technique and the selection of suitable preparations at both 4μ and 8μ for comparing tissues before and after treatment. The pretreatment and posttreatment preparations of both the normal and affected sites were stained for carbohydrate-containing proteins by the McManus-Hotchkiss method § simultaneously in the same container to avoid possible variations in staining technique.

METHODS OF TREATMENT

The 15 patients selected for study were divided into the following groups for therapy. Eight were treated with corticotropin. Seven of these later received a course of treatment with oral hydrocortisone. Three of the seven were subsequently also treated with cortisone. Six of the seven had, at another time, topical treatment with hydrocortisone ointment. These various courses of treatment were given at intervals of not less than two months to permit hormonal readjustment and the return of the dermatitis to its severe state.

The seven patients who were not given corticotropin received either cortisone or hydrocortisone. Following are the details of the treatment:

1. Corticotropin gel was given as a single daily injection, 100 to 160 units for either one or two days, followed by 80 units daily for four to seven days, and finally 40 to 60 units daily until the second biopsy specimen was made. The dose was then gradually reduced over a two-week period before corticotropin was discontinued. The criteria for the dosage and duration of treatment before the second biopsy was the same for corticotropin, cortisone, and oral hydrocortisone. The severity of the condition determined the initial dose, the clinical course the subsequent doses. The second biopsy specimen was made after the patient had shown marked improvement and when no further improvement in the condition was noted for three successive days. The dose thereafter was gradually reduced during another two weeks before treatment was discontinued. The interval between the first and second biopsies varied from 10 to 14 days.

2. Cortisone was given in a 200 mg. divided dose daily for one or two days. Thereafter, the dose was reduced to 80 mg. daily for another four to seven days and, if improvement was marked, to 60 mg. daily. The second biopsy specimen was made after 10 to 14 days of treatment, and the dose was gradually reduced over a two-week period before cortisone was discontinued.

3. Hydrocortisone was given in divided doses of 160 mg. daily for one or two days. It was then reduced to 80 mg. daily and maintained as with cortisone until the second biopsy specimen was made at the end of 10 to 14 days. Thereafter, the dose was reduced slowly as with cortisone.

The three groups described received 2 gm. daily of enteric-coated tablets KCl in divided doses.

4. Topical therapy with 2.5% hydrocortisone acetate (Merck) was used in six of the patients. Two corresponding sites which appeared morphologically similar were selected, either on each arm or on opposite sides of the chest. The patient was instructed

† In their study of ground substances with the McManus-Hotchkiss method, Gersh and Catchpole [18] characterize the material as follows: "Intercellular substances fall into at least two categories. The first of these includes various substances summarized by the term 'tissue fluid': water containing proteins, crystalloids, metabolites, and gases. These are related primarily to the plasma, whose composition they largely reflect, and only secondarily to connective or other tissues. The second category includes the microscopically non-fibrillar ground substance(s) whose most characteristic component is a glycoprotein (that is a protein which contains a carbohydrate moiety as an integral part of its structure)."

‡ References 2 and 3.

§ References 2 and 3.

to rub the ointment gently into one of the sites over an area about 2.5 cm. in diameter for three minutes, three times daily. At the end of seven days biopsy specimens were taken from the untreated site and from the center of the treated area.

RESULTS

Clinical Findings

The improvement of the skin condition was marked before the end of a week in all patients treated with corticotropin, oral cortisone, and oral hydrocortisone. Generally, itching became negligible after the first 24 hours of treatment. Thereafter, progress varied with the chronicity and degree of lichenification. Patients with long-standing, severe, papular lesions required a longer period of treatment and a higher dose than those with acute lesions and less lichenification. After therapy was discontinued, the dermatitis gradually returned to its original severity. The importance of decreasing the dose slowly in discontinuing treatment was demonstrated in one patient who had an acute flare-up of a generalized atopic dermatitis involving the face, neck, trunk, arms, and thighs, when the dose of hydrocortisone was reduced too rapidly. This patient stated that dermatitis had never previously been present elsewhere than on her arms, face, and neck. The condition responded to an increased dose of hydrocortisone, which was gradually reduced over a period of four weeks. Since then, however, she has had a tendency to a more diffuse distribution of her atopic dermatitis than had been present before hydrocortisone treatment.

The six patients who used hydrocortisone ointment topically all showed marked improvement at the treated site. Four of these had definite improvement at all other areas of atopic dermatitis, though not to the degree of the treated site. The reason for this general improvement was not clear. It hardly seemed likely that the amount of hormone absorbed either from the treated site or from the handling of the ointment could have produced the improvement at otherwise untreated areas. The improvement of the dermatitis in areas distant from the site of treatment has been attributed by some observers to inadvertent spread of the ointment [5] and by others || to systemic absorption of the hormone.

Histological Findings

Pretreatment Tissues.—The histopathologic findings as shown by routine stains in untreated atopic dermatitis have been well described, especially by Heimann [8] and Alexander.[9] Briefly, in all our patients the epidermis showed marked acanthosis with moderate to severe intercellular and intracellular edema (Fig. 1*A*). The degree of edema was especially noticeable because the method of freeze-drying eliminates most of the cell shrinkage that occurs with chemical fixation. The epidermal cells were markedly swollen and destroyed in some areas, producing the condition termed "*altération cavitaire*." The intercellular spaces were greatly widened. Acanthosis was present in variable degrees in all cases.

The dermis, especially in the papillary and upper portions, was highly vascularized and edematous. There was a generalized increase in cellular content, especially of fibroblasts, chromatophores, and histiocytes. In addition to the generalized increase in connective tissue cells, all of the sections showed a marked perivascular concentration of the cells noted above and a variable number of round cells of the lymphocytic type. Many of the histiocytes were multinucleated (Fig. 1*A*, *p*). Eosinophiles were irregularly distributed even in the same tissue, being present in variable numbers in some, and completely absent in other, sections.

Post-Treatment Tissues.—The changes after treatment (Fig. 1*B*), as observed with hematoxylin and eosin stains, were very similar following the various hormones used, except that the alteration was not as great in the patients who were treated topically. The epidermal cells resembled those of normal tissue in size. The intercellular spaces of the epidermis likewise were reduced to

|| References 6 and 7.

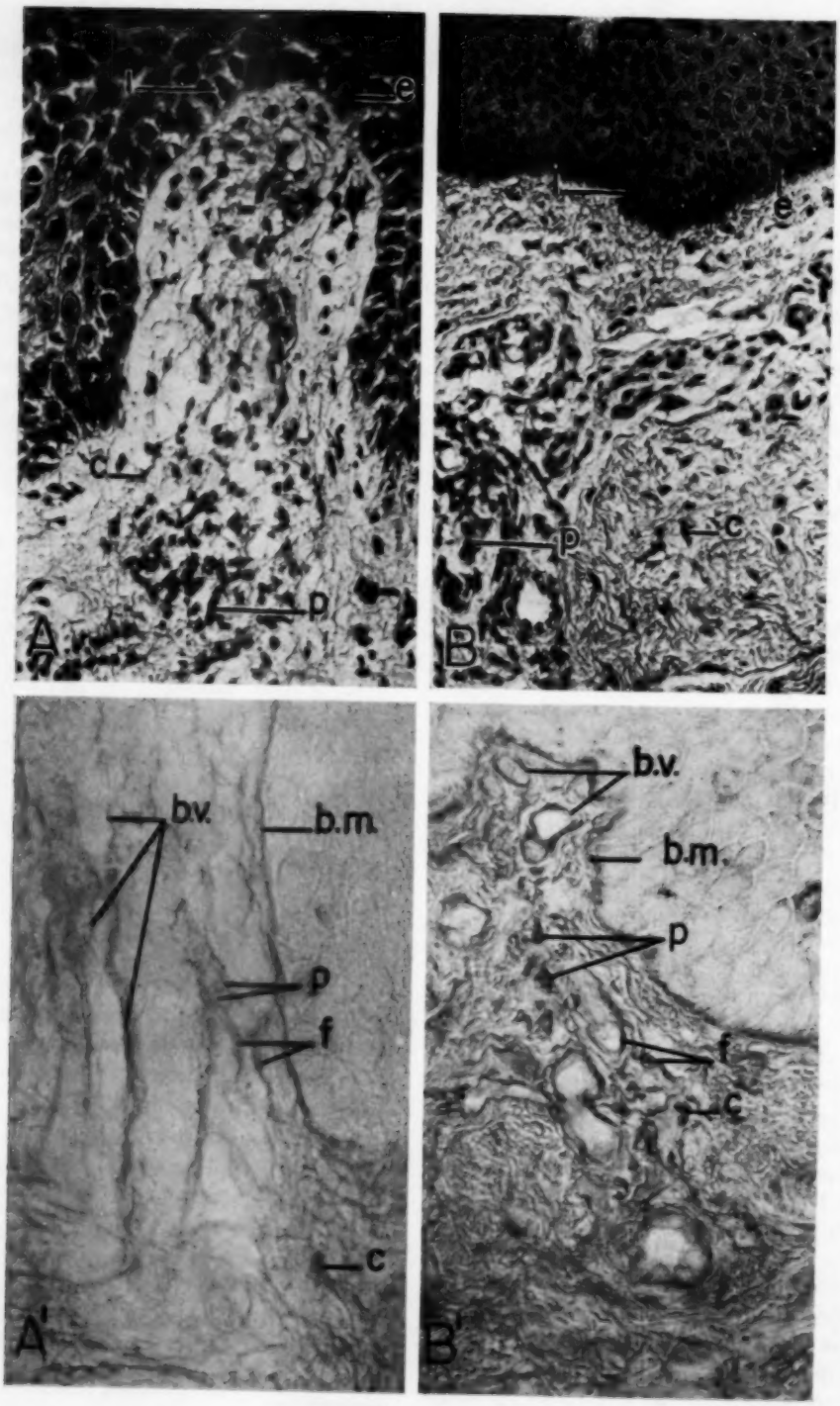

Figure 1

(See legends on opposite page)

normal. No change occurred in the acanthosis due to proliferation of the stratum germinativum.

The dermis, after treatment, showed, with the routine stains, a reduction in the edema. The fibrous tissue was now generally organized in bundles rather than as separated fibers. Some perivascular edema persisted in many tissues. While there was no change in the number or distribution of the cells in the connective tissue, the reduction of the edema produced an apparent increase in cellular infiltration (Fig. 1*A* and *B*).

No difference was found in the skin from "normal" areas when pre- and post-treatment tissues were compared.

Histochemical Findings

Pretreatment Tissues.—When stained with the McManus-Hotchkiss method, the epidermis contained purple, intracellular granules, frequently in clumps. These could be digested with β-amylase, indicating that the material was glycogen.

The dermis showed the following with the McManus-Hotchkiss staining method: The subepidermal basement membrane was thinner and stained less distinctly in the tissues from untreated atopic areas, as compared to normal tissues from the same patient (Fig. 1*A*,[1] *b. m.*). This change was greater where the edema of the cutis was more acute. With the oil immersion lens the basement membrane of normal tissue appeared to be a homogeneous material, staining more deeply than the adjacent ground substance and containing fibrils whose long axes were parallel to each other in the basement membrane. In tissues from patients with untreated atopic dermatitis, the homogeneous material stained much more lightly, and the included fibers had lost their uniform orientation and frequently lay diagonally to the direction of the basement membrane. In addition, in the tissues from sites of active dermatitis, the subepidermal basement membrane appeared as a straight thin "line" (or sheet), while in normal skin the basement membrane appeared as a deeply stained, relatively wider zone with a sharply dentated juncture between basal cells and basement membrane. In normal skin this junction gave the appearance of interdigitations of the basement membrane into the bases of the basal cells. The loss of these sharp indentations in the atopic tissues seemed to be related to the epidermal edema.

The perivascular basement membranes were similarly thin and poorly stained, as compared to normal tissue. In the more acute clinical alterations, the edema of the cutis was severe and the changes around the vessels were marked. Indeed, in some sites, the perivascular membranes were hardly visible (Fig. 1*A*,[1] *b. v.*).

The ground substance had a reduced stainability which also paralleled the degree of edema. This change was present in all layers of the cutis but was most marked in the upper and middle zones. The loss of stainability of the ground substance was particularly evident in the perivascular areas. The fibrous

Explanation of Figure 1

Fig. 1.—Sections from adjacent areas of acute atopic dermatitis before (*A* and A^1) and after treatment with corticotropin (*B* and B^1). All four sections were photographed at the same magnification (× 600).

In the hematoxylin and eosin stained sections (*A* and *B*), compare the following in the pretreatment and post-treatment sections: the wide interepidermal spaces (*i*) in *A* due to interepidermal edema, the difference in size of the epidermal cells (*e*) before and after treatment due to the difference in edema of the cells, the difference in the dermal edema in *A* and *B*. Note also the multinucleated perivascular polyblasts (*p*) and the chromatophores (*c*).

In the McManus-Hotchkiss preparations of the same tissues (A^1 and B^1) the following comparisons should be noted: the difference in the thickness and staining intensity of the subepidermal (*b.m.*) and perivascular (*b.v.*) basement membranes. In A^1 they are thinner and stain less than in normal skin. In B^1 these structures stain as in normal skin. The ground substance in A^1 stains less intensely than in B^1. The fibroblasts (*f*) and polyblasts (*p*) in B^1 stain more deeply than in A^1.

Chromatophores (*c*) are observed in both A^1 and B^1.

tissue was separated into individual fibers in the more edematous tissue, and the fibers were more dispersed where the edema was greater.

The fibroblasts in tissues from untreated atopic areas had two distinctly different staining characteristics, depending on their location (Fig. 2, f_2 and f_1). Those grouped about the blood vessels stained much more deeply than adjacent fibroblasts. Fine, cytoplasmic granules were seen in many of these cells. In addition to fibroblasts, the perivascular groupings contained lymphocytic-type round cells, chromatophores, and polyblasts. The chromatophores contained yellow to brown melanin granules. The mononucleated and polynucleated histiocytes contained purple-staining granules of uniform size which were larger than those of the fibroblasts.

Differentiation between the numerous histiocytes (polyblasts) and the relatively few eosinophiles, whose granules stain similarly with the McManus-Hotchkiss method, was accomplished in a number of selected tissues by comparing adjacent, thin sections stained respectively with the McManus-Hotchkiss and Giemsa methods.¶

The lighter-staining areas adjacent to the perivascular zones contained, in addition to numerous fibroblasts, a variable number of histiocytes and chromatophores.

Post-Treatment Tissues.—The tissues taken from atopic areas after treatment with corticotropin, cortisone, and oral hydrocortisone all showed uniform and similar changes. The difference observed in patients treated topically with hydrocortisone ointment will be discussed separately.

The epidermis contained a similar amount of glycogen to that in untreated tissues. The dermis showed the following changes: The subepidermal basement membrane of areas, after treatment, approached in stainability and thickness that of normal tissue from the same patients. The junction between basal cells and basement membrane had in most cases regained the dentate appearance of the corresponding normal tissues (Fig. 1*B*,[1] *b. m.*). Under oil immersion the fibers in the deeply staining, homogeneous matrix had regained uniform orientation, parallel with the basement membrane, characteristic of normal skin.

The perivascular basement membranes in the atopic areas following treatment had also

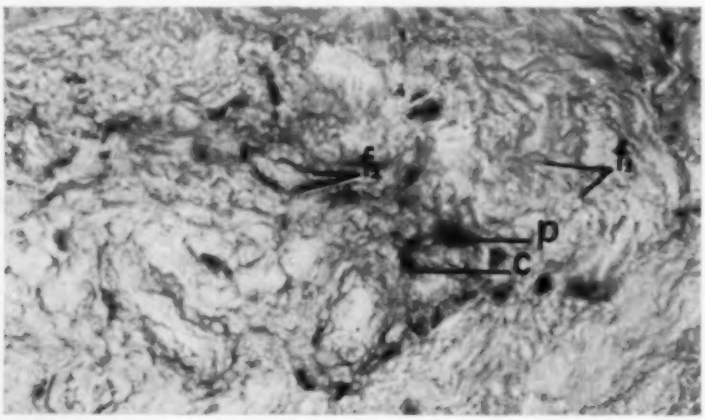

Fig. 2.—This section, stained by the McManus-Hotchkiss method, shows a perivascular zone and the surrounding area. Note the difference in staining intensity between the fibroblasts (f_2) in the perivascular zone of infiltration and those in the adjacent peripheral area (f_1). Many chromatophores (c) and a few multinucleated polyblasts (p) are shown in the perivascular zone.

¶ Since mast cells also have large granules which stain similarly with the McManus-Hotchkiss method, the mononucleated histiocytes had to be differentiated from these as well. Examination of sections stained with toluidine blue showed that only an occasional preparation contained a few mast cells.

regained their stainability and thickness, resembling those in normal tissues (Fig. 1*B*,[1] *b. v.*).

The ground substance in the post-treatment tissues likewise stained more deeply, resembling the normal matrix in the same patients. Lighter-staining zones were observed, in some cases, only in perivascular regions, where the edema and loss of stainability had been greatest.

The distribution and type of connective tissue cells in the skin after treatment resembled that before treatment, but their stainability was in general greater than that in pretreatment tissues. This was particularly evident in the fibroblasts, both in the perivascular and in the surrounding zones.

The tissues from normal sites showed no differences when pretreatment and post-treatment sections were compared.

Topical Treatment.—The sites treated topically with hydrocortisone ointment, when compared with the corresponding untreated areas, showed similar changes, as did those treated with the other substances. However, the change after such treatment was less marked than in those treated with corticotropin, cortisone, and oral hydrocortisone. The most distinct alteration was a reduction in the epidermal and dermal edema. The changes in the stainability of the basement membranes and ground substance could be observed by careful comparison of treated with untreated areas.

COMMENT

The changes that are demonstrable during the active state of atopic dermatitis by the McManus-Hotchkiss method consist of increased edema of the epidermis and dermis and decreased stainability of the ground substance, basement membranes, and fibroblasts. Following treatment with corticotropin and adrenocortical steroids, the staining characteristics of the skin resemble those of normal tissue: the edema is reduced; the ground substance stains with normal intensity; the basement membranes resemble those of normal tissue, and the fibroblasts stain more deeply than those in the skin before treatment.

The basis for these changes can be understood from recent studies,# which indicate that normal connective tissue is organized as a heterogeneous physicochemical system containing negatively charged colloid. The mucoproteins of the ground substance form a substantial part of this colloid and determine its behavior. At the simplest level there would be two phases in equilibrium, a colloid-rich, water-poor phase and a water-rich, colloid-poor phase. The two-phase system is, of course, equilibrated with the blood.

The work of Gersh and Catchpole [13] and of others * demonstrates that ground substance is not inert, but is a labile material whose composition can change rapidly. Thus, while the matrix in normal skin consists predominantly of material in a colloid-rich state, the shift in balance to the water-rich phase may occur quickly.

In atopic dermatitis two alternate mechanisms, not necessarily mutually exclusive, may explain the changes from a predominantly colloid-rich to the water-rich phase of the connective tissue and account for the changes in stainability. First, it is possible that, under altered conditions, the cells secreting mucoproteins produce a colloidal material which is more water-soluble. Such altered secretion would result in a shift of ground substance toward the water-rich phase. A second hypothesis, offered by Gersh and Catchpole,[13] is that the labile colloids of ground substance can either become "depolymerized," breaking down ultimately to water-soluble material, or can, under other conditions, become "polymerized" into larger aggregates.

Since the McManus-Hotchkiss procedure has a high degree of specificity for carbohydrate-containing substances, these changes in the state of the ground substance can be directly related to the stainability of comparable sections. In the early stages of pathologic change due to depolymerization, there

References 10, 11, and 12.

* References 14 and 15.

may be exposure of an increased number of reactive radicals which, after oxidation with periodic acid, combine with the fuchsin stain used, with a resulting increase in stainability of the ground substance. If the break-down of the highly polymerized glycoproteins progresses, or if the synthesis is altered so that an abnormal amount of water-soluble material is formed, a change in balance from the colloid-rich to the water-rich phase may result. To maintain osmotic equilibrium, fluids would diffuse into the tissues, and water-soluble mucoproteins and electrolytes, into the blood. The resulting loss of stainability of the tissue with the McManus-Hotchkiss method in that case would be due not only to a loss of carbohydrate-protein conjugates but also to the dilution effect of the edema.

The decreased stainability and thinning of the basement membranes are, presumably, also due either to altered synthesis or to break-down of glycoproteins. These changes are not confined to the allergic state. They have been noted also in such varied conditions as scurvy,[16] inflammation,† tissue adjacent to growing tumors,‡ desquamative gingivitis,[18] and ulcerative colitis.[19]

Meyer,[20] and Gersh and Catchpole [13] have suggested that fibroblasts may be the source of ground substance or its precursor. This was inferred from the presence of deep-staining Hotchkiss-positive granules in fibroblasts at sites of active tissue changes. In atopic dermatitis, the deeply stained perivascular fibroblasts with cytoplasmic granules and the numerous histiocytes (polyblasts) containing deeply stained granules of uniform size suggest active secretion by these cells either of ground substance or its precursors. Similar granules in the sinusoidal cells of regenerating liver were observed by Aterman.[21] The reduced stainability, with the McManus-Hotchkiss method, of the fibroblasts adjacent to the perivascular infiltrations in tissues before corticotropin or corticoid therapy, as compared to that in treated tissues, may depend on the rapid destruction of ground substance and the need for its speedy discharge from the cells that produce it; or, possibly, on an exhaustion of the capacity of the fibroblasts to produce mucoproteins to meet the rapid and abnormal demand. Conversely, the increased stainability of fibroblasts and histiocytes following corticotropin and corticoid treatment, as compared with those in pretreatment tissues, may be due to the decrease in break-down of ground substance, so that more of the stainable material can accumulate within the cells, or to the formation of a different type of material which stains more deeply with the McManus-Hotchkiss method.

Decreased permeability and increased tonicity of blood vessels after corticosteroids has been noted by a number of workers.§ The increased stainability of the dermis and thickening of the basement membrane, following treatment with corticotropin, cortisone, and hydrocortisone, may be related to these findings. This pattern is consistent with a shift in the tissue balance from the water-rich to the colloid-rich phase. On the basis of the two explanations previously suggested, the effect of the therapy may be to restore or stimulate the capacity of cells to produce "normal" mucoprotein or to retard the speed of mucoprotein break-down.

SUMMARY AND CONCLUSIONS

During active atopic dermatitis the following changes occurred.

1. The ground substance and the basement membranes stained less intensely than in normal skin with the McManus-Hotchkiss method. These changes were interpreted as evidence of an altered state in the glycoproteins of the ground substance and basement membranes and a loss of soluble material through the blood vessels.

2. The cytoplasm of the fibroblasts stained less deeply in the untreated atopic skin.

3. Many deep-staining granules of uniform size were present in the histiocytes. These latter two observations were interpreted as evidence of exhaustion of secretory

† References 13 and 14.

‡ References 13 and 17.

§ References 22 and 23.

function in fibroblasts and the maintenance of such function in the more primitive histiocytes.

Following treatment with corticotropin, cortisone, and hydrocortisone, the atopic skin showed the following connective tissue changes:

1. The ground substance and basement membranes were stained deeply by the McManus-Hotchkiss method, resembling the normal skin in these respects.

2. The cytoplasm of the fibroblasts and histiocytes stained more deeply in the tissues after treatment. These two changes were interpreted as due to an arrest, or decrease, in the rate of break-down of mucoproteins, and possibly also to stimulation of the secretory function of connective tissue cells.

Dr. Frederick K. Heath, of Merck & Company, supplied the cortisone acetate ointment (2.5%) used in the topical treatment of six of the patients.

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Responses of the Liver to Injury

Effects of Cortisone upon Acute Carbon Tetrachloride Poisoning

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The serial changes of the cytoarchitecture, nucleic acids, and nitrogen of the rat liver following an acute injury with carbon tetrachloride have been studied by histological, spectrophotometric cytochemical, and chemical methods.[1] The patterns of the alterations have served as references for the investigation of the effects of compounds known to influence tissue inflammation and regeneration. This report deals with the effects of cortisone upon this acute hepatic injury. Cortisone alters profoundly the repair of this lesion.

METHODS

Male Wistar rats weighing about 200 gm. were fed and caged as described earlier.[1] For two days prior to the single intraperitoneal injection of CCl_4 (0.066 cc. in liquid petrolatum/100 gm. body weight), they were given 20 mg. of cortisone acetate, intramuscularly, daily. This dose was continued until the time of killing, 24, 48, 72, 96, and 120 hours after CCl_4 administration.

The techniques for the several methods of analysis have been reported in detail.[1]

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RESULTS

Clinical.—While all animals survived the experimental procedures, those receiving cortisone showed marked increased irritability and lost considerable amounts of weight (Table 1).

Histological.—Control, No CCl_4 (Plate I, Figs. 1*a* and 1*b*). The liver cells of the control, untreated rats showed the usual coarse basophilic clumps scattered diffusely throughout the cytoplasm (Plate I, Fig. 1*a*). No fat was seen in these liver cells.

The pericentral liver cells of animals which received cortisone for 48 hours without CCl_4 (Plate I, Fig. 1*b*) showed scattered basophilic cytoplasmic clumps. However, especially in those cells about the portal vein, the basophilia was noted chiefly at the cell wall, while the perinuclear area was clear. Fat droplets were seen in all of the liver cells and were more marked in those cells about the portal vein. The scattering of basophilic clumps to the cell walls was not related to the spatial distribution of the fat droplets.

Twenty-Four Hours After CCl_4 (Plate I, Figs. 2*a* and 2*b*): The livers of both groups of rats showed an equally wide zone of centrilobular necrosis (Plate I, Figs. 2*a* and 2*b*). The surviving periportal liver cells of the controls showed a loss of cytoplasmic basophilic clumping with vacuolation. On the other hand, the periportal cells of the cortisone-treated group showed basophilic clumping about the cell membrane. These latter cells were similar in appearance to the periportal liver cells in rats that had received cortisone without CCl_4 (Plate I, Fig. 1*b*). Fine fat droplets were seen throughout the liver cells of both groups, especially in the necrotic cells.

TABLE 1.—*Analyses of Liver Tissue*

Groups, Hours After CCl_4	No. of Rats	△Body Wt., Per Cent	Organ Wt., Gm.	SEm	Per Cent Body Wt.	Per Cent Dry Wt.*	SEm	DNAP γ/Mg., Dry	SEm	DNAP Mg./100 Gm., Wet	SEm	RNAP γ/Mg. Dry	SEm	RNAP Mg./100 Gm., Wet	SEm	Nitrogen Mg./Mg., Dry	Nitrogen Gm./100 Gm., Wet
0	28	0	8.01	.14	4.00	21.1	.47	.85	.01	18.30	.31	4.57	.10	96.92	1.94	.131	2.76
0 + Cortisone	10	− 9.9	8.30	.56	4.42	16.2	1.10	.79	.03	12.55	.83	4.33	.16	69.06	5.20	.121	1.96
24	10	− 6.5	9.43	.39	4.76	20.5	.71	.88	.02	17.99	1.58	4.80	.17	97.53	5.85	.129	2.65
24 + Cortisone	5	−10.0	10.11	.26	5.50	17.2	1.39	.80	.03	13.85	1.10	4.55	.10	78.16	3.16	.125	2.16
48	23	− 2.3	9.84	.22	4.34	17.4	.56	1.40	.03	23.76	.81	6.20	.11	106.96	3.32	.128	2.23
48 + Cortisone	8	−15.4	8.82	.23	5.90	16.8	.36	.70	.01	11.77	.48	4.05	.20	68.27	3.17	.120	2.01
72	9	− 4.0	10.40	.44	4.93	15.1	.83	1.15	.05	17.06	1.25	4.68	.16	84.62	3.58	.131	1.98
72 + Cortisone	12	−16.7	8.52	.49	4.82	16.5	.64	.88	.01	14.65	.56	4.74	.08	79.06	2.69	.118	1.95
96	10	− 3.5	9.07	.53	4.07	20.2	.38	.92	.05	19.33	1.34	4.87	.21	96.66	3.94	.128	2.59
96 + Cortisone	5	−17.4	11.07	.80	6.16	17.0	1.54	.93	.02	15.80	.81	4.48	.06	76.20	6.38	.129	2.20
120	10	+ 1.0	9.51	.38	4.18	21.7	.50	.92	.04	16.85	.43	4.59	.14	99.78	3.96	.124	2.69
120 + Cortisone	5	−18.7	9.07	.85	5.42	17.7	.81	.81	.04	14.50	.71	4.25	.13	75.60	3.46	.137	2.42

* Residual dry weight after cold-TCA and alcohol-ether extraction.

Forty-Eight Hours After CCl_4 (Plate II, Figs. 3*a* and 3*b*): The control animals showed marked inflammatory cell infiltration of the wide necrotic zone, and peripheral to this there were about five mitoses per high-power field in the surviving periportal cells (Plate II, Fig. 3*a*). The nuclei of the surviving cells were enlarged and their cytoplasm contained many vacuoles. There was very little coarse basophilia in the cytoplasm.

The livers of the cortisone-treated group showed a wide zone of centrilobular necrosis, relatively free of inflammatory cells (Plate II, Fig. 3*b*). The surviving periportal cells contained basophilic clumps at the basement membranes, as well as some vacuoles. The nuclei were small and there were only one or two mitotic figures per high-power field. Fat stains were similar in both groups and resembled those of animals 24 hours after CCl_4.

Seventy-Two Hours After CCl_4 (Plate II, Figs. 4*a* and 4*b*): The livers of the cortisone-treated rats could be divided into two groups. One group of six was similar to the cortisone-treated animals 48 hours after CCl_4 (Plate II, Fig. 3*b*). In the second group of six animals, the zone of necrosis was completely filled with orderly arranged liver cells. One or two mitotic figures were seen per high-power field. The surviving cells were similar in appearance to the periportal cells of rats receiving cortisone alone (Plate II, Fig. 4*b*).

In contrast, the control animals had not reached this latter stage of healing at 72 hours. The histological appearances of these livers were more uniform than were those of the cortisone-treated group. Mitotic activity was rare. While the necrotic cells had cleared, the central area was not completely restored with new liver cells but contained inflammatory cells, enmeshed in a latticework of reticulum (Plate II, Fig. 4*a*). The fat stains showed fine fat droplets in the liver cells of both groups of animals.

Ninety-Six Hours After CCl_4 (Plate III, Figs. 5*a* and 5*b*): The livers of cortisone-treated animals (Plate III, Fig. 5*b*) were completely restored and were similar to those

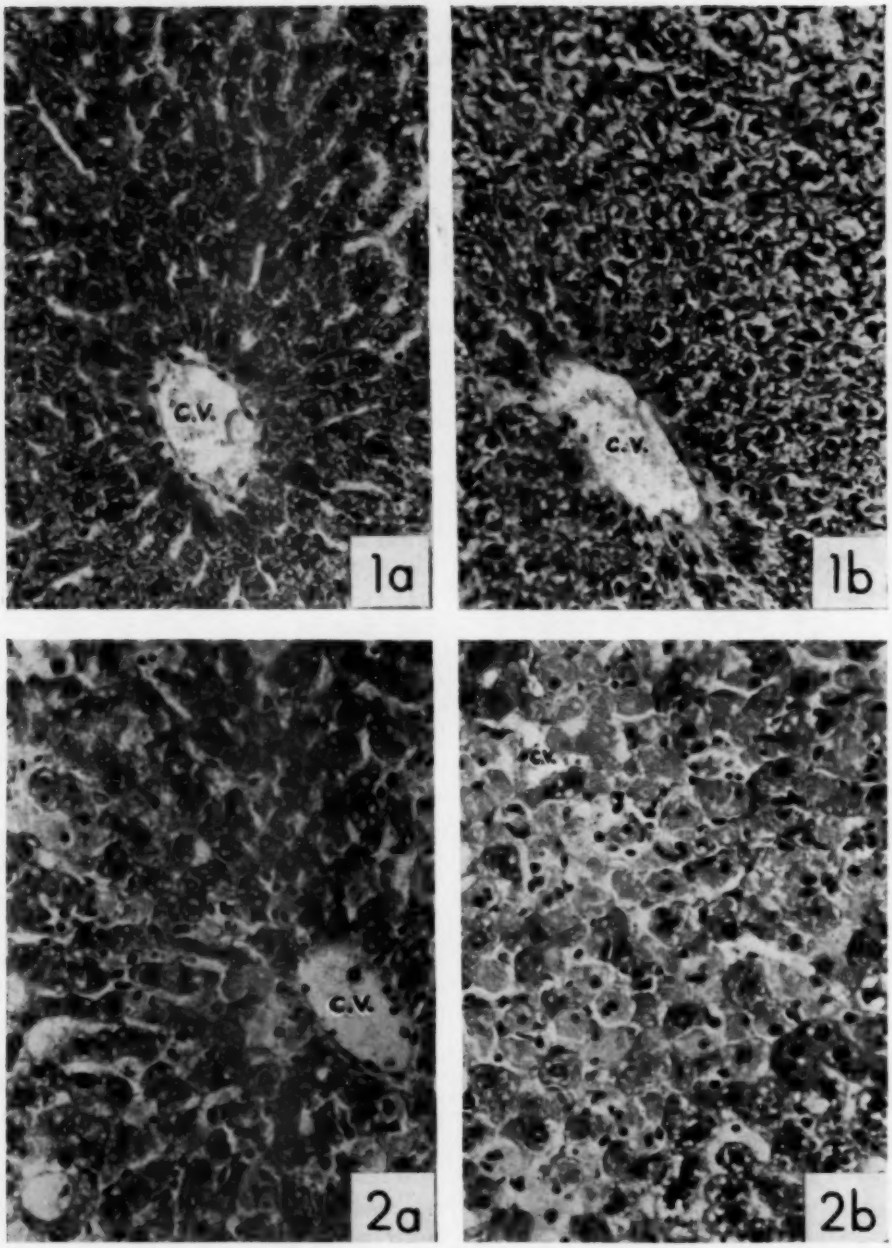

Plate I

Giemsa stain; magnification × 252 in all photomicrographs. C. V. indicates central vein.

Fig. 1*a*.—Normal control. The coarse basophilic cytoplasmic clumps are outlined throughout the liver cells.

Fig. 1*b*.—Control, cortisone. The perinuclear space is clear and the cytoplasmic basophilic clumps are about the cell wall.

Fig. 2*a*.—Control, 24 hours after CCl_4. There are necrosis and vacuolation of liver cells about the central vein.

Fig. 2*b*.—Cortisone, 24 hours after CCl_4. There are necrosis and vacuolation in the pericentral zone.

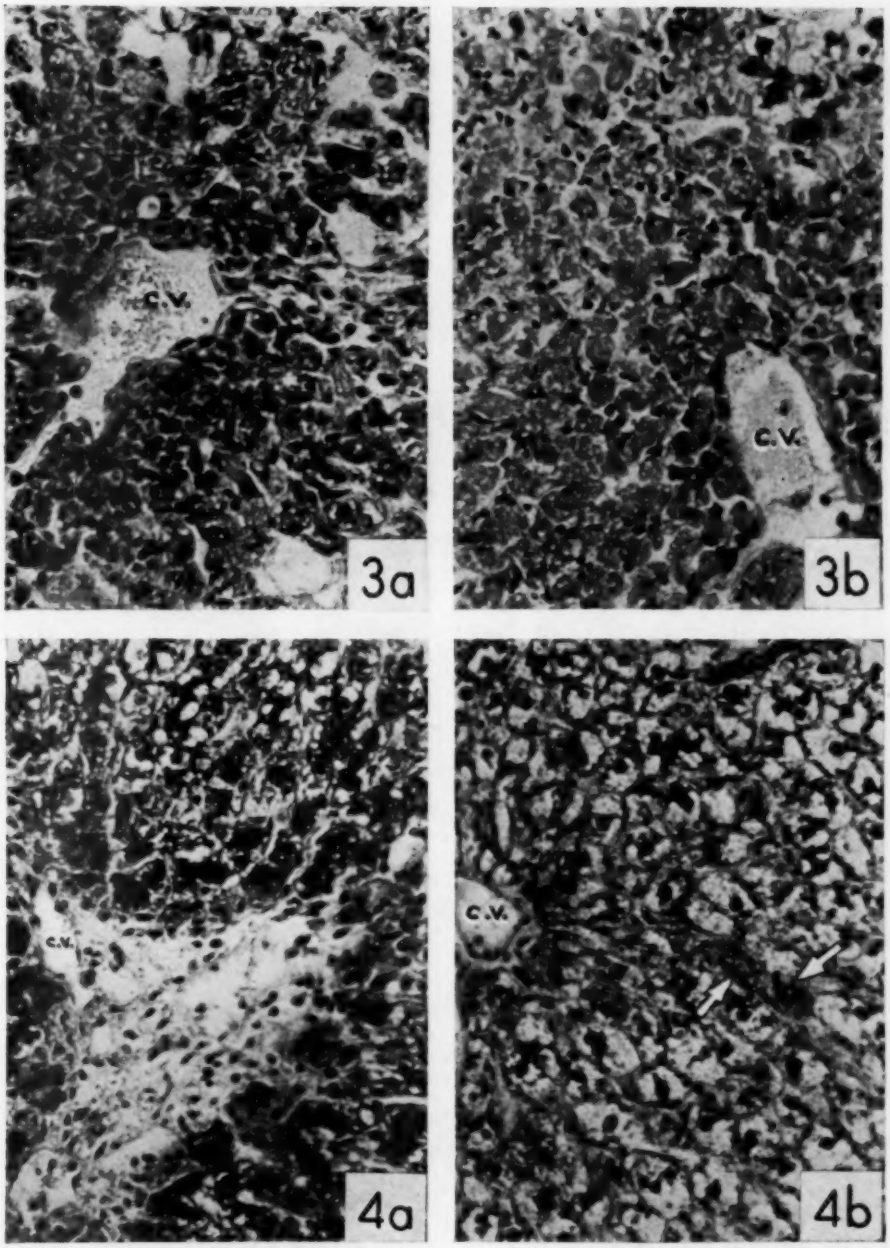

Plate II

Fig. 3*a*.—Control, 48 hours after CCl_4. The pericentral zone of necrosis is heavily infiltrated with inflammatory cells. The mitotic figures are not seen in this photograph, since they are in surviving cells peripheral to this zone.

Fig. 3*b*.—Cortisone, 48 hours after CCl_4. The pericentral zone of necrosis is relatively free of inflammatory cells. In this section the surviving cells are not seen.

Fig. 4*a*.—Control, 72 hours after CCl_4. The pericentral zone contains not only new liver cells but also a lacework of connective tissue infiltrated with inflammatory cells.

Fig. 4*b*.—Cortisone, 72 hours after CCl_4. The pericentral zone has been restored with new liver cells. Two mitotic figures are indicated by Arrows, among the surviving cells.

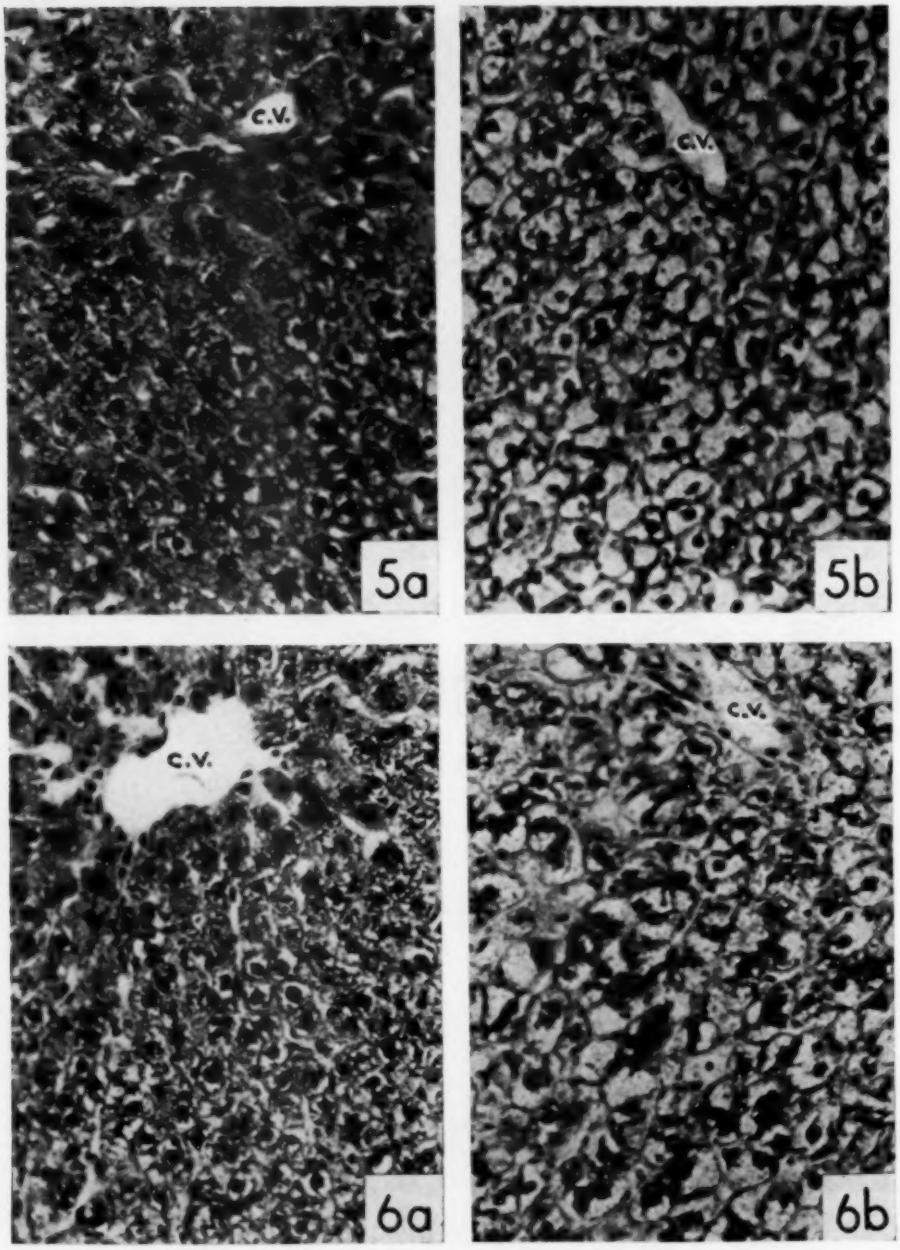

Plate III

Fig. 5*a*.—Control, 96 hours after CCl_4. There are a few inflammatory cells remaining in an otherwise restored pericentral zone.

Fig. 5*b*.—Cortisone, 96 hours after CCl_4. Restoration is complete. The liver cells appear similar to those in Plate I, Fig. 1*b*.

Fig. 6*a*.—Control, 120 hours after CCl_4. Restoration is complete. The liver cells appear similar to those in Plate I, Fig. 1*a*.

Fig. 6*b*.—Cortisone, 120 hours after CCl_4. Same as Fig. 5*b*.

of animals which had received cortisone but no CCl_4 (Plate I, Fig. 1*b*). The control livers showed rare mitotic figures and occasional inflammatory cells in the pericentral zone (Plate III, Fig. 5*a*). Fat droplets were diffusely distributed throughout the livers of the cortisone-treated animals. On the other hand, in the control rats, fat droplets were confined to an occasional pericentral liver cell.

In summary, the cortisone-treated livers differed from the controls in that they showed (*a*) changed appearance of the cytoplasmic basophilic granules, (*b*) reduced mitotic activity, (*c*) reduced inflammatory cell infiltration, (*d*) accelerated healing in some animals, and (*e*) increased amounts of stainable fat in the liver cells.

Spectrophotometric-Cytochemical.—While the frequency distributions of nuclear classes,

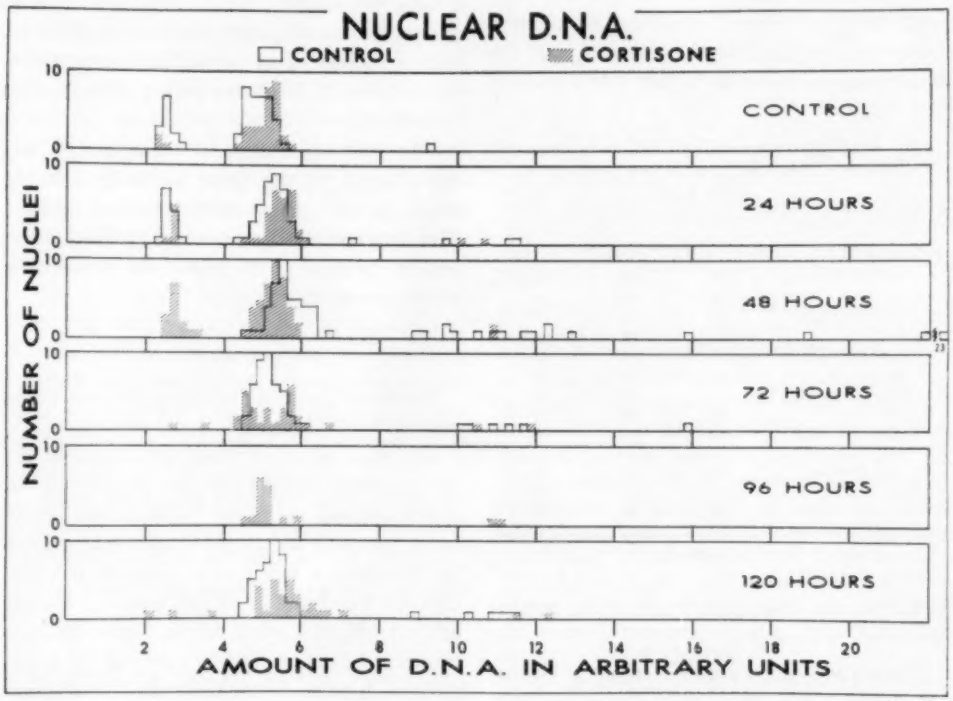

Plate IV

This histogram shows the frequency distributions of nuclear classes according to amounts of nuclear DNA, expressed in arbitrary units. It is evident that the shift to higher classes is more marked in the control group than in the cortisone-treated group. The persistence of abnormal frequency distribution at 120 hours is seen in both groups.

One Hundred Twenty Hours After CCl_4 (Plate III, Fig. 6*a* and 6*b*): The livers of each group were the same as their respective controls. Fat droplets were still scattered throughout the liver cells of the cortisone-treated livers. However, only rare fat droplets were seen in the pericentral zones of the livers of control animals.

as determined by nuclear deoxyribonucleic acid (DNA) estimates,[1] were markedly altered in both groups of animals, striking differences were observed between the control and cortisone-treated groups (Plate IV and Table 2).

The control group showed a small shift at 24 hours and the maximal shift to higher nu-

clear classes at 48 hours. Thereafter there was a trend back toward the starting values. The frequency distribution at 120 hours was still abnormal.

While the cortisone-treated group showed a shift to higher classes at 24 hours and later, at no time did the shift to higher classes equal that noted in the control group. On the other hand, at 120 hours the frequency distribution was still abnormal, as in the control group.

The administration of cortisone was associated with a reduced build-up of nuclear DNA as compared to the controls, but the occurrence of nuclei of higher DNA content

TABLE 2.—*Frequency Distributions* of Hepatic Cell Nuclear Classes in Control and Cortisone-Treated Rats*

Groups, Hours After CCl_4	Nuclear Classes		
	2 ×	4 ×	8 ×
0	.26*	.73*	.02*
0 + Cortisone	.22	.77	.01
24	.23	.73	.04
24 + Cortisone	.16	.82	.01
48	.01	.75	.24
48 + Cortisone	.07	.90	.03
72	.02	.89	.09
72 + Cortisone	.09	.83	.07
96	...	...	...
96 + Cortisone	.08	.88	.03
120	.01	.86	.13
120 + Cortisone	.03	.92	.05

* In per cent of 200 nuclei counted.

at 120 hours after CCl_4 was similar to that observed in controls.

Chemical.—The marked rise in tissue DNAP and RNAP (dry and wet weight), which occurred at 48 hours after CCl_4 in the control livers, was absent in the cortisone-treated livers (Table 1). Indeed, the nucleic acids (dry weight) in this latter group remained unchanged throughout the experiment. Small but significant increments in nucleic acids per unit wet weight were noted at 72 and 96 hours in the cortisone-treated groups.

The nitrogen content (dry weight) remained unchanged in the control group. In the cortisone-treated group the nitrogen content was somewhat lowered at the beginning and remained at this reduced level until 96 hours and 120 hours after CCl_4, when it rose.

The marked fluctuations in tissue dry weight which were seen in the control livers were not observed in the cortisone-treated group. Here the per cent of dry weight started 23% below the starting value for the control animals and fluctuated very little.

The liver increased in size in both groups. In the control group this increase was sustained, reaching a peak at 72 hours and declining thereafter. In the cortisone-treated group, there was a sharp increase at 24 hours, with a falling off at 48 and 72 hours, and a second rise at 96 hours and a falling off at 120 hours.

The striking feature of these studies was the absence of the large increments in the nucleic acids in the cortisone-treated animals. The lowered nitrogen content of these livers and its increase at the end of the experiment were noteworthy.

COMMENT

Cortisone produced striking changes in the reparative phases of this acute hepatic injury. There was no effect upon the extent of necrosis, even though the fat content of the organ seemed increased.[2] There was an almost complete ablation of inflammatory cell proliferation which was so prominent a feature in the controls at 48 hours. Mitotic activity was at a much reduced level but continued for longer periods (present 72 hours after CCl_4) than in the controls. The changes in nuclear DNA were less marked than in the control groups but were similar qualitatively.

The tissue nucleic acids content was not increased markedly during active regeneration, as was the case in the controls. Some of this, particularly as concerns DNAP, might be attributed to the absence of large numbers of inflammatory cells. However, the significant increase in tissue RNAP (at 48 hours) observed in control livers did not occur in the cortisone-treated animals. The initially reduced tissue nitrogen levels [3] increased after cytoarchitectural restoration had occurred.

There are several noteworthy features to these studies. First, under the influence of cortisone administration, the clearing of necrotic tissue was accomplished in the absence of a heavy infiltration with inflammatory cells. This finding indicates that the enzymes required for the digestion and disposal of necrotic tissue are present, either in necrotic tissue, in the extracellular fluid bathing this tissue, and/or the surviving cells adjacent thereto, and that these enzymes need not be derived solely from inflammatory cells. These results differ from those reported in other systems of experimental necrosis, where cortisone delayed healing.* This discrepancy may be related to the particular noxious agent which was employed in each experimental design. On the other hand, Lattes and co-workers [9] have shown that the initial inflammatory and reparative responses were not altered by cortisone.

Second, the cytoarchitectural restoration of the liver occurred at a level of mitosis and nucleic acid synthesis which was reduced much below that of the controls. In spite of this, in some cortisone-treated rats, the degree of healing achieved at 72 hours exceeded that found in the control group.

These studies reaffirm the well-established concepts of the anti-inflammatory † properties of cortisone, as well as its inhibitory effects upon mitosis and nucleic acid synthesis.‡ These depressing effects upon nucleic acid synthesis offer a suggestion for the decreased mitotic activity of the regenerating organ and, at least in part, a possible basis for the anti-inflammatory effect. In this connection, the studies of Green and co-workers § and of Bullough ‖ are of considerable interest. These authors have suggested that cortisone, as well as other antimitotic agents, operate by inhibiting the cellular oxidative carbohydrate metabolism. Indeed, Bullough [18] has indicated that cortisone inhibits hexokinase activity.

Studies are continuing of the effects of other forms of stress, as well as of the possible relationships of adrenal, hypophyseal, and gonadal secretions in this experimental system.

SUMMARY

Cortisone had a marked effect upon the healing of the hepatic lesion produced by acute CCl_4 injury. It reduced mitosis, inflammation, and nucleic acid synthesis. However, healing proceeded to completion without delay.

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News and Comment

GENERAL NEWS

Dedication of the Armed Forces Institute of Pathology.—The dedication ceremonies for the Armed Forces Institute of Pathology were held in Washington, D. C., on May 26 and 27, with President Eisenhower giving the dedication address. In his address the President remarked that, "Some years ago those of us who advocated unification, saw something like this in the offing." The Institute serves as the central laboratory for all the armed services, the Veterans Administration, Public Health Services, Atomic Energy Commission, and other agencies. The President was introduced by Brig. Gen. Elbert DeCoursey, the Institute director.

In the evening of May 26, Dr. W. M. Stanley, of the University of California, gave the scientific dedicatory address. On Friday the scientific program of the dedication ceremonies was held. In the morning session Dr. Arnold Rich was the presiding officer, and in the afternoon, Dr. Howard T. Karsner. The following papers were presented:

Sunlight as an Environmental Factor in Cancer of the Skin, Harold F. Blum, Ph.D., Princeton University

The Relationship of Senile Elastosis to Actinic Radiation and to Carcinoma of the Lip, Col. Joseph L. Bernier, D.C., A.F.I.P.

The Problem of Metabolic Response to Injury, Paul R. Cannon, M.D., University of Chicago

The Spectrum of Structural Changes in Acute Renal Failure, Jean R. Oliver, M.D., Emeritus Professor of Pathology, State University of New York

The Physiologic Disturbance in Acute Renal Failure, John P. Merrill, M.D., Peter Bent Brigham Hospital

Wound Ballistics, Capt. William M. Silliphant (M.C.) U. S. N., Deputy Director, A. F. I. P.

The Battle Wound, John H. Howard, M.D., Baylor University

Decompression Sickness, Capt. Albert R. Behnke (M.C.) U. S. N., Radiological Medical Director, San Francisco

The Pathology of Chronic High Altitude Anoxia, Alberto Hurtado, M.D., Research Director, Institute of Andean Biology, Lima, Peru, S. A.

Decompression Sickness, with Special Reference to Flying, Webb E. Haymaker, M.D., A. F. I. P.

The new A. F. I. P. building, ground for which was broken in July, 1951, was built at a cost of more than $7,000,000. It is a monolithic concrete blast-resistant structure with heavily reinforced walls, 12 to 16 inches thick. It has eight stories, five above ground and three below. It has an area of 130,000 net square feet, and the main central mass, exclusive of the two expandable wings, measures 206 × 102 feet. It is completely air-conditioned, with fluorescent lighting throughout. The laboratories are of modular construction, each unit measuring 11 × 20 feet arranged along a central core where they are connected with piped laboratory services.

Keratoacanthoma (Molluscum Sebaceum)

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and
HERMAN PINKUS, M.D., Detroit

In the last few years references to self-healing squamous-cell epithelioma or keratoacanthoma have become numerous in the dermatological literature. In a recent review and case report [1] the authors considered keratoacanthoma (molluscum sebaceum), tumor-like keratosis of Poth,[2] and the solitary and multiple self-healing tumors of the Ferguson Smith type [3] as variants of one entity. Prior to 1934, when Ferguson Smith described a case of multiple, primary, squamous-cell carcinoma with spontaneous healing, all of these single or multiple lesions were considered to be malignant epidermoid carcinoma. In the following two decades more clinicians came to recognize this skin lesion. Dermatologists learned that certain tumors, which had the histologic characteristics of low-grade epidermoid carcinoma, underwent spontaneous healing or disappeared after biopsy or incomplete removal. Correlation of the clinical impression and histologic material is necessary for diagnosis but has been a matter of confusion in many cases.

This paper purposes to review the recent literature and present the histologic features of a number of representative cases of keratoacanthoma in order to clarify the diagnosis of these histologically malignant but clinically benign lesions.

CLINICAL FEATURES

Some dermatologists state that keratoacanthoma resembles squamous-cell cancer clinically. Levy and co-workers [4] describe a keratoacanthoma as a small papular lesion that enlarges rapidly to maximum size in four to eight weeks. The fully developed lesion is a firm nodule 1 to 2 cm. in diameter (Fig. 1). The overlying skin may be shiny but at times is erythematous. The lesion presents a central crater filled with keratin material. Occasionally the crater is covered by a scale. Regression begins spontaneously in about six to eight weeks from the onset, and involution is completed within four to six months. A depressed puckered scar may remain.

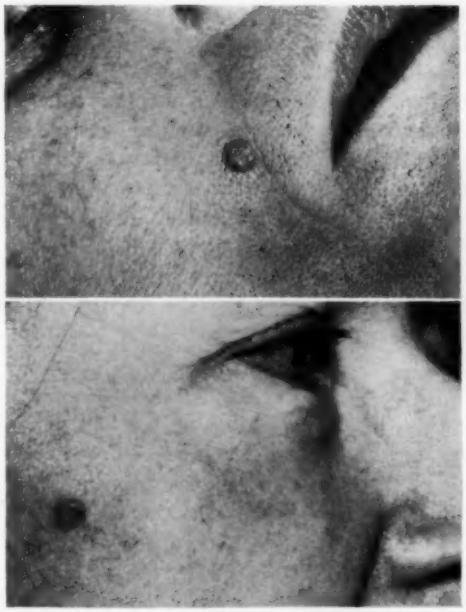

Fig. 1.—Upper photograph is the lesion in Case 2. The lower photograph is from Case 4.

A superficial resemblance of small lesions to molluscum contagiosum accounts for the term "molluscum sebaceum" so frequently used in the English literature interchangeably with keratoacanthoma. Even under low-power magnification gross sections may suggest the pattern of molluscum contagiosum.

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Clinically, most writers in this field agree that the rapid appearance and growth of the skin lesion, usually on the face when solitary without associated lymphadenopathy, is a valuable point in distinguishing keratoacanthoma from squamous-cell cancer. Beare [5] believes the crater containing well-formed keratin is a distinguishing point from other skin lesions that could be confused with keratoacanthoma. Weidman [6] attempts to predict cancer versus keratoacanthoma when the gross specimen is bisected preparatory to histological embedding. He states that the cut surface in keratoacanthoma reflects the keratinous crater and the good localization exhibited clinically, and the bottom parts are found to be more or less streaked (keratinized). In cancer, the crater is not as disproportionately huge and the texture in the bottom parts is homogeneous like a raw potato.

For the purpose of illustrating the histopathologic criteria for diagnosis, we have chosen four cases of a keratoacanthoma to show some of the slight variations in pattern.

REPORT OF CASES

Case 1.—Mrs. C. C., 78 years, W. F., a diabetic for nine years. A lesion considered to be a sebaceous cyst formed on the left side of the nose during January, 1954, and increased rapidly in size. In March, it was approximately 2 cm. in diameter. This nodule was seen in April by one of us (H. P.). At this time there was a semiglobular, firm, keratotic tumor of the left ala. Its sides were covered by smooth epidermis, and its diameter was 1.2 cm. Upon considering the history, the clinical diagnosis of keratoacanthoma was made. Under local anesthesia most of the tumor was excised and the base then curetted and fulgurated. Healing took place rapidly under the crust. Three months later, in July, 1954, a slightly depressed smooth scar was present. The patient was last seen on Jan. 15, 1955. There was no sign of recurrence or metastasis.

Case 2.—Mrs. R. A., 725839, 50 years old. Patient had multiple lesions over the face, neck, and back resembling seborrheic keratoses and cellular nevi. In 1953 one lesion was removed from the face (seborrheic keratosis) and one from the back (benign pigmented nevus). In December, 1954, she was seen at Henry Ford Hospital dermatology department. She had a slightly rough-surfaced half-spherical lesion of 7 mm. diameter over the right cheek just lateral to the lower end of the nasolabial fold. This nodule was of seven to eight weeks' duration and was surrounded by an erythematous halo (Fig. 1). Clinically this lesion resembled a molluscum contagiosum. It was removed under local anesthesia by curettage. The skin over the area is well healed.

Case 3.—Mr. N. R., 58 years, W. M., works on Michigan Road Commission. He received a spatter burn from hot oil over the thenar surface of the right hand on Nov. 11, 1954. The skin did not heal. The lesion was lanced some weeks later by a physician. Swelling increased after this procedure. The patient was first seen Jan. 14, 1955, by a dermatologist (H. P.). The tumor was 2 cm. in diameter and dusky red in color. The margins were covered with smooth epidermis, and the center was keratotic. This nodule was excised with a narrow margin under local anesthesia. The clinical impression was keratoacanthoma. The incision healed promptly.

Case 4.—Mrs. G. C., 80313, 60 years old. The patient, first seen on Dec. 16, 1954, had a 7 mm. lesion over the right zygoma. The nodule was firm and had a rolled border and a depressed central area. Clinically it resembled either a basal-cell epithelioma or a molluscum contagiosum (Fig. 1). The tumor was removed by curettage and the base treated by electrodesiccation. The area shows good healing.

HISTOPATHOLOGICAL COMMENT

The histologic differentiation between keratoacanthoma and the so-called self-healing squamous-cell epithelioma of the skin may be difficult, if not impossible.* Just as difficult is the more important differentiation from true progressive carcinoma. A biopsy specimen of almost all of these lesions will appear as a keratinizing epidermoid carcinoma, histologic Grade 1 (Broders). Ormsby and Montgomery [9] believe that one of the cases reported by Sommerville and Milne [10] is a Grade 2 pseudoepitheliomatous hyperplasia. It must be emphasized, however, that most of these tumors are truly invasive and destroy normal tissue (Fig. 2). They may extend downward through the full thickness of the corium, and elastic fiber stains often show that the invasive epithelium engulfs and destroys elastic fibers.

In each suspected clinical case of keratoacanthoma the tumor should be bisected so that the keratin mass will be included in the

* References 7 and 8.

Fig. 2 (Case 3).—This is an example probably of the most typical pattern of keratoacanthoma. Similar photographs are seen in the articles in the American literature. Note the overhanging of the normal epithelium at the margin of the keratotic plug and the deep invasion of the corium.

section. Generally, on very low-power examination the pattern of the lesions resembles molluscum contagiosum but on a much larger scale. The keratohyaline layer is prominent in most instances along the ragged base of a crater filled with keratin and parakeratotic material. The underlying epithelium is very acanthotic. There are many papillary projections of the epithelium similar to pseudoepi-

Fig. 3 (Case 3).—Higher-power view shows invasion and epithelial pearls. Note the dense lymphocytic infiltration. Reduced about ⅓ from mag. × 100.

Fig. 4 (Case 4).—The degree of exocytosis is prominent in this higher-power area. This is a field showing unusual thickening and overgrowth of squamous elements. Reduced about ⅓ from mag. × 100.

Fig. 5 (Case 1).—Higher magnification of portion of the tissue. Note the epithelial pearls and well-differentiated squamous cells. Reduced about ⅔ from mag. × 100.

theliomatous hyperplasia (Fig. 3). The basal layer is generally intact except where invaded by infiltrate (Fig. 4). Hyperchromatic nuclei are common (Fig. 5). Mitotic figures are present in varying numbers. Throughout the epidermis there are many cells showing individual keratinization and larger "pearl formation," but most of the epidermis will consist of well-differentiated squamous cells showing intercellular bridges (Fig. 6). A mononuclear infiltrate is generally present around the base. This infiltrate should be present as an exocytosis; i. e., the wandering

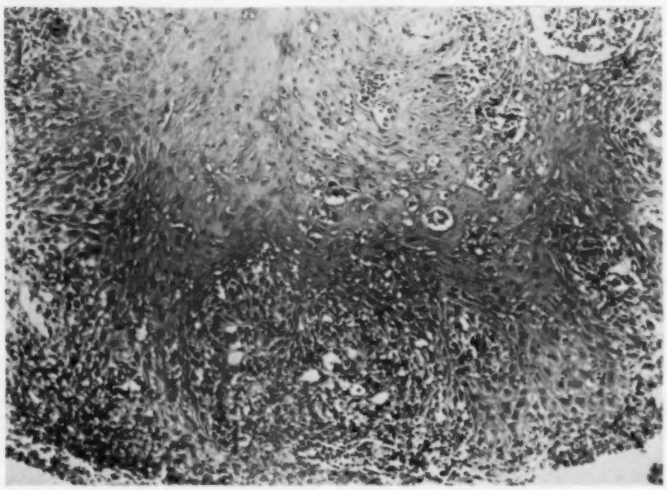


Fig. 6 (Case 2).—The epithelial elements are well differentiated even in the downward projections which are invading the dermis. Reduced about ⅓ from mag. × 100.

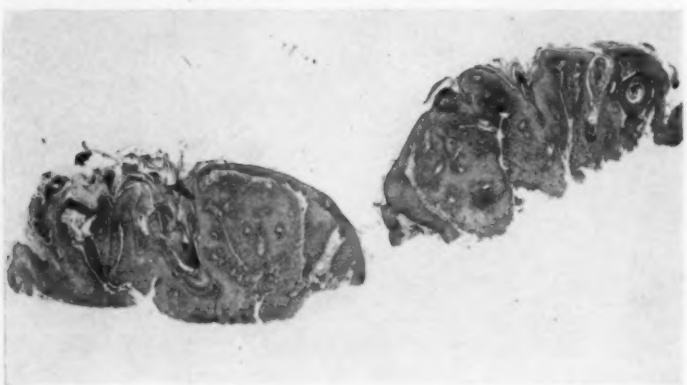


Fig. 7 (Case 2).—Histopathologic section shows the keratotic plug. The section was not bisected exactly. Note the downward projections of epithelial elements. Reduced about ⅓ from mag. × 12.

Differential Points of Keratoacanthoma and True Malignant Skin Lesions

Keratoacanthoma	Squamous-Cell Carcinoma
1. May resemble molluscum contagiosum	1. Usually does not resemble molluscum contagiosum
2. Rapid growth	2. Generally slower growth
3. Large keratin plug in a crater	3. A single large plug generally absent
4. Extremely friable surface	4. Variable friability
5. Exocytosis	5. Lack of exocytosis
6. Basal-cell layer intact	6. Basal-cell layer not intact
7. Overhanging of normal epithelium	7. Often absence of overhanging epithelium

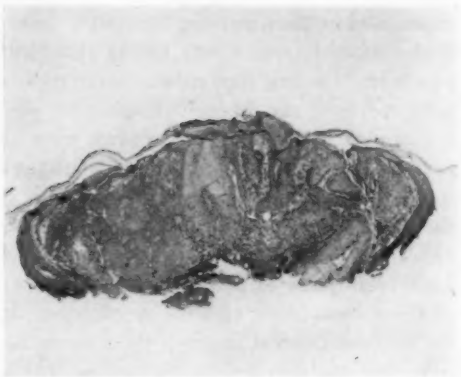


Fig. 8 (Case 4).—Low-power view of biopsy section. There is normal squamous epithelium over both margins but not a good marginal buttress formation. Reduced about ⅓ from mag. × 12.

cells have migrated up through the epithelium rather than appearing as if the squamous cells have pushed aside the infiltrate (Fig. 4).

One histologic feature that has been emphasized in the literature [14] as characteristic of keratoacanthoma is the marginal buttress formation (Fig. 2). This results from the fact that invasive epithelium undergrows the normal epidermis and pars papillaris of the surrounding skin, which, in turn, overhangs the central crater. This feature, however, may be found as well in epidermoid carcinomas of varying degrees of malignancy and appears dependent solely on the gross type of growth of the individual lesion. While it is apparently a constant factor in keratoacanthoma, it can be recognized only in favorable sections through the center of the tumor but not in tangential sections (Figs. 7 and 8), or in small biopsy sections of a large lesion. One histologic finding appears certain, and that is the fact that a suspected keratoacanthoma never goes beyond the state of a Grade 1

Fig. 9 (Case 1).—Histopathologic section shows a relatively late stage in which the keratinized mass is being sequestered leaving a deep defect in the skin. Reduced about ⅖ from mag. × 12.

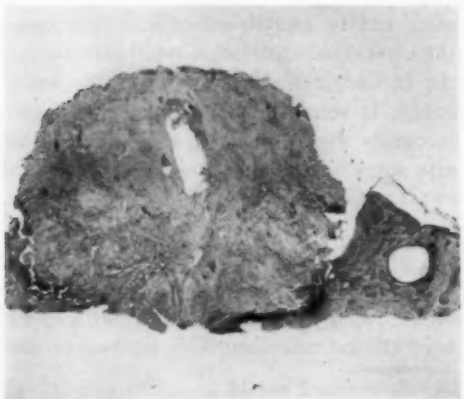
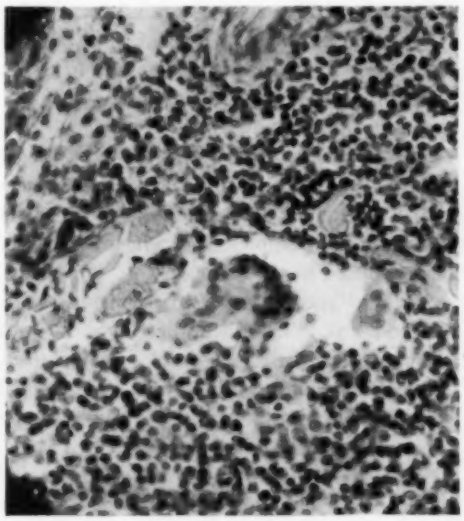


Fig. 10 (Case 1).—Showing foreign-body reaction at base of lesion. This appears to be an attempt at beginning healing. Reduced about ⅓ from mag. × 430.



prickle-cell carcinoma in appearance, in contrast to the clinical history of rapid development over a few weeks.

Some of the differential points between keratoacanthoma and true malignant skin lesions are given in the accompanying Table.

In the later stages of development, the entire tumor may be seen to become separated from the surrounding tissue (Fig. 9) and even larger portions of its mass consist of keratin, including remnants of collagenous and elastic fibers. Some degree of foreign-body reaction may develop around the deep parts of the lesion (Fig. 10). Eventually the tumor becomes sequestered, leaving a defect that heals with more or less scarring.

ETIOLOGY

These self-healing tumors have been considered to arise from hypertrophic and inflammatory changes in sebaceous cysts.[11] Poth [2] indicated sunlight and an unknown factor as the cause of the tumors he described. New and Horton [12] also believe that sunlight is the causative agent. Genetic influence has been emphasized by Sommerville and Milne [10] and Charteris [13] in the cases of Ferguson Smith epithelioma. Ereaux and co-workers [1] believe a virus may be responsible for the lesion. They report a case associated with recurrence of herpes simplex on the lips. They promulgate trauma as an agent, as there were in excess of 100 active lesions and scars which developed on the extremities of this same patient following gasoline burns. To date, virus transmission of this entity remains to be proved. Binkley and Johnson [14] relate keratoacanthoma to aging when it occurs on the skin of the face, ears, or hands as a single lesion. They describe a second type (industrial) that may be multiple, occurring on areas of skin exposed to oil or tar.

Becker [15] mentions a case described by Witten and Zak,[16] in which there was a poral closure of the sebaceous and sweat glands. He suggests that this might have an etiological bearing. The initial eruption in this instance was miliaria.

In 1953 Marshall and Findlay [7] suggested that the self-healing property of these tumors may be due to their derivation from the hair apparatus. They compare the extrusion of the tumors after a time to that of hairs from the hair follicle.

COMMENT

Following Ferguson Smith's report of self-healing squamous epithelioma numerous other examples began to appear in the world literature, but for almost two decades none were mentioned in the American literature. In the first reported cases (six) in the American literature [4] the first four patients were treated with roentgen ray therapy (4000 r) or electrosurgery. The next two patients were allowed to go untreated and spontaneous resolution occurred. A biopsy was performed in each of the latter cases. Skirpan and Haserick [17] reviewed the clinical and histopathologic findings of 14 cases in their paper. All of these cases were treated by excision. In the two most recent papers † the authors believe that excision and cautery of the base is the best form of therapy. These papers report seven cases of keratoacanthoma, including one with multiple lesions of 19 years' duration.[1] Included in one paper [14] are three keratoacanthomas, two of which were multiple, on skin exposed to tar or oil. However, Beerman [18] cautions against including such malignant and premalignant processes as oil keratosis in the group of keratoacanthomas.

There have been enough cases now reported in the world literature that to mention an exact number is unnecessary. There is little question that these cases have been frequently seen and misdiagnosed. Definite diagnosis can be established only by combining the clinical information of rapid growth with the histologic findings of low-grade malignancy. It seems important that pathologists recognize these lesions so that they can advise surgeons to avoid unnecessarily radical measures in treatment.

SUMMARY

Keratoacanthoma is probably a good name for the rapidly growing nodules with a superficial clinical resemblance to molluscum con-

† References 1 and 14.

tagiosum, which often are suspected clinically to be carcinomas. Some factors have been mentioned which should help the pathologist differentiate the lesion from the typical, low-grade, squamous-cell carcinoma of the skin.

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Infectious Pancreatic Necrosis in Brook Trout

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and
W. T. YASUTAKE, B.A., Cook, Wash.

During the winter and spring of 1954 several outbreaks of mortality were observed among fingerling brook trout in the eastern United States. These occurred soon after feeding of the fry started. The symptoms were characteristic for "acute catarrhal enteritis." * Some of the symptoms also resembled octomitiasis, but losses continued even after treatment for Octomitus was successful. Observations described in this article indicate that on the basis of histopathological examination the disease should be classified as an infectious pancreatic necrosis.

Pancreatic necrosis in mammals is generally credited to the action of pancreatic enzymes which are liberated from the ducts. The aberrant distribution of the enzymes has been attributed to a number of conditions including any anatomical arrangement which allows infected bile to flow into the main pancreatic duct.[3] Obstructions of small ducts within the pancreas may also lead to the condition,[4] or the initiating factor may be a mild bacterial infection or a focus of infarction in the pancreas due to thrombosis of diseased vessels.[5] Pappenheimer and coworkers[6] were the first workers to describe a specific pathogen for pancreatic necrosis. They reported that Coxsackie virus (Connecticut-5 strain) produced a highly selective pancreatic disease in adult mice. The present paper is a note on an additional infectious agent which produces specific pancreatic necrosis in brook trout.

Submitted for publication Feb. 16, 1955.

Salmon Nutrition Laboratory, Cook, Wash., and Microbiological Laboratory, Leetown (P. O. Kearneysville) W. Va.

* References 1 and 2.

MATERIALS AND EXPERIMENTS

In January, 1954, a disease occurred in the fingerling brook trout population at the Fish and Wildlife Service Station, Leetown, W. Va. The most characteristic symptom of the disease was a very violent whirling and corkscrewing motion, which suggested that the affected fish were literally writhing with pain. Mortality of infected lots in some cases reached 75%.

Grossly, the fish appeared normal with the exception of petechiae in the vicinity of the pyloric caeca. Routine examinations for bacteria and parasites were negative. Histologically, however, extreme pancreatic necrosis was observed.

The infected fish were in two groups from unrelated parent stock. In the first group, from Bellefonte, Pa., the epizootic started earlier, reached a peak in 10 to 12 days, then subsided over the continuing six-week period (Fig. 6). In the second group, from Berlin, N. H., the mortality started two weeks later, reached a peak in 10 to 12 days, and subsided somewhat more rapidly.

To test for transmission of the disease by water, fingerling brook trout were obtained from a third source of unrelated parent stock at Beaver Creek, Md. A trough was partitioned into two sections. The infected Berlin group was placed in the upper section of the trough and 565 of the Beaver Creek group were placed in the lower section. Typical symptoms of the disease developed in the exposed group 6 days later, and the mortality reached a peak in 10 to 14 days. Acute pancreatic necrosis was observed in both groups histologically.

In July, the disease occurred in two West Virginia hatcheries at Dorcas and Marlington. The infected fish showed typical symptoms of the disease; the mortality curve reached a peak in 10 to 14 days, and acute pancreatic necrosis was observed histologically. At the time of writing this paper (January, 1955) reports indicate that the disease may be even more widely spread this year.

PATHOLOGY

Gross Lesions.—An unusual feature of this disease is the extremely high mortality

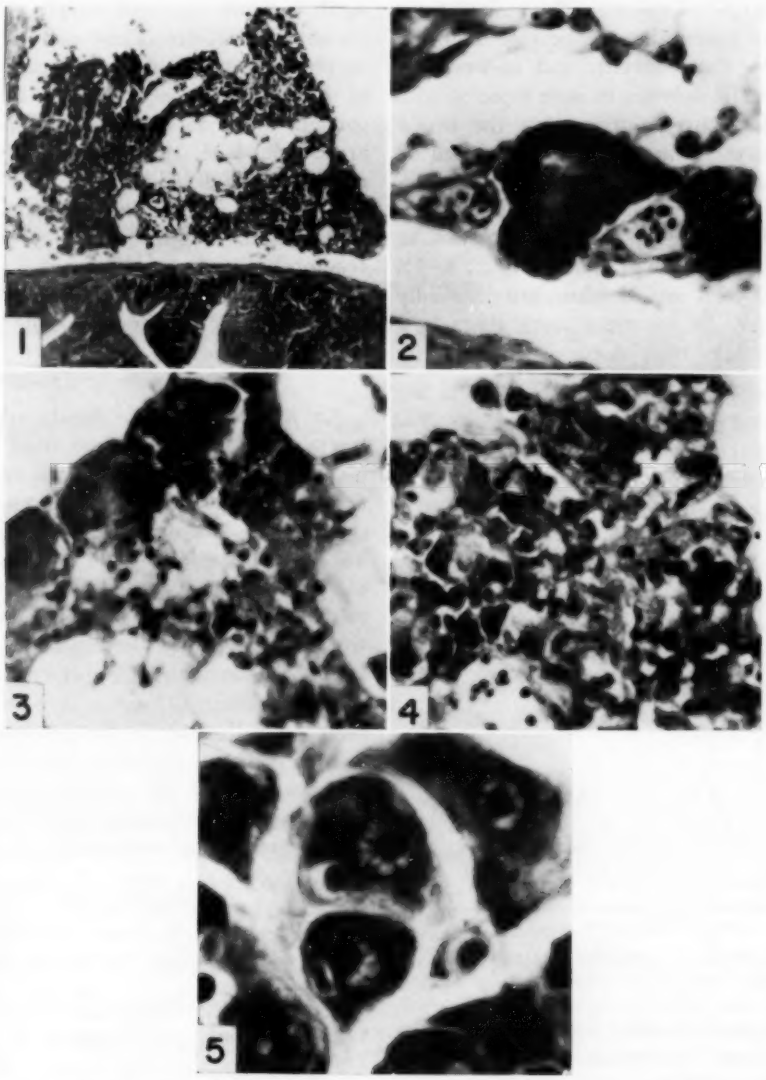

Fig. 1.—Necrotic pancreatic tissue adjacent to a pyloric caeca. Hematoxylin and eosin; × 100.

Fig. 2.—An unaffected foci of acinar cells. Hematoxylin and eosin; × 400.

Fig. 3.—A relatively early stage of the disease showing the edge of the advancing necrosis. Hematoxylin and eosin; × 400.

Fig. 4.—An advanced stage of the necrosis. Hematoxylin and eosin; × 400.

Fig. 5.—Inclusion bodies in acinar cells at the edge of the advancing necrosis. Hematoxylin and eosin; × 1350.

which occurs with practically no grossly observable lesions. Small petechiae in the vicinity of the pyloric caeca are the only visible abnormalities. In fish, the pancreas is not a distinct, encapsulated organ. The acinar tissue and islets are scattered throughout the mass of pyloric caeca, which are grouped about the small intestine immediately below the juncture with the stomach. The minute hemorrhages occur in this tissue.

Microscopic Lesions.—The microscopic picture is practically identical to that described by Pappenheimer and co-workers[6] for pancreatic necrosis in mice injected with Coxsackie virus. In many fish the major portion of the acinar tissue is obliterated by a massive necrosis. The entire acinar structure is replaced by a mass of necrotic detritus containing fragmented acinar cells, pyknotic nuclei, and zymogen granules (Figs. 1 to 4). The cytoplasm and detritus are markedly eosinophilic. In advanced cases there is a marked cellular reaction consisting primarily of monocytes but with a few polymorphonuclears present. Fat deposits, which are commonly found infiltrating and surrounding the pancreatic tissue of hatchery fish, show an extensive fat necrosis, presumably from the lipolytic enzymes. In contrast to Pappenheimer's observations, however, the islet tissue is not entirely spared. Not infrequently, extensive necrosis also occurs within the islets. Small ducts within the necrotic areas usually appear unharmed.

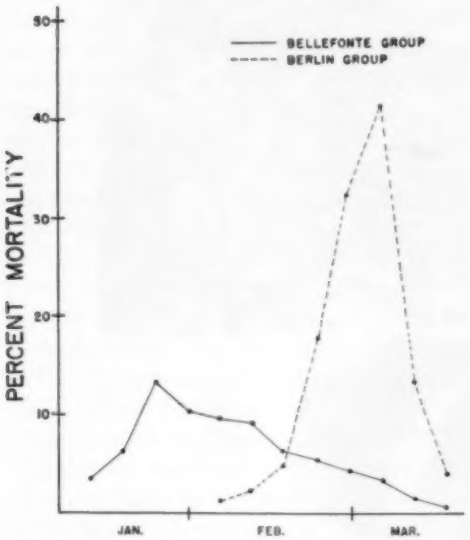

Fig. 6.—Per cent mortalities calculated for five-day periods.

The initial stage of the necrosis does not start from a single focus of infection. Instead, small foci are scattered widely throughout the acinar structure. These rapidly encroach on the surrounding tissue. At the periphery of these areas of necrosis many acinar cells contain intercytoplasmic inclusions. These are small, blue, usually oval bodies surrounded by a clear halo (Fig. 5). The definite location of these inclusions at the edge of the advancing necrosis may indicate a relationship to the causative agent.

No other organs show any significant changes, grossly or histologically.

COMMENT AND SUMMARY

An infectious disease highly specific for the pancreas of young brook trout has been observed. The lesions are those of massive necrosis of the acinar and islet tissues. The complete absence of bacteria or parasites in infected fish suggests that a virus may be the causative agent. The similarity of the lesions to those described by Pappenheimer and co-workers[6] following the injection of Coxsackie virus in adult mice also lends credence to this possibility. Neither is it inferred at the present time nor is the possibility overlooked that the inclusion bodies observed in acinar cells at the periphery of necrotic areas are true virus inclusion bodies. Further studies are in progress to clarify the etiologic agent.

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Arteriosclerosis in the Cat

Naturally Occurring Lesions in the Aorta and the Coronary Arteries

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Studies on the pathogenesis of arteriosclerosis in a variety of species have been under investigation in this laboratory. In the aorta and coronary arteries of the dog * and in the muscular arteries of the bird,† the initial manifestation of naturally occurring arteriosclerosis consists of degeneration of the elastic tissue—a process that is soon followed by intimal thickening and fibrosis. Interestingly enough, these early changes in the artery are not associated with lipid infiltration of the intima. The experimental induction of a lipemia may, however, result in an infiltration of lipids in the preexisting, naturally occurring lesion and at times gives rise to a new lesion that is primarily lipid in origin.

Arteriosclerosis in the cat has received little attention. Fox stated that Felidae seldom have vascular disease,[6] but he did observe intimal granulations of the thoracic and abdominal aorta similar to those found in dogs. Fox also noted that the feline aortic intima did not become a wide layer until full maturity or old age was reached.

The present report is based on the examinations of hearts and aortas of 36 cats, 14 of which were 5 years of age or older. This study stresses the similarity in the degenerative vascular lesions of natural origin among birds, dogs, human beings, and cats and again emphasizes the absence of lipid material in the earliest arterial lesions of another species, the cat.

MATERIALS AND METHODS

Thirty-six cats were used in this study. The first 18 (Table) were obtained from the county animal shelter and were of unknown age. Their size and appearance, however, suggested that the majority were young animals. The other 18 cats were obtained from several veterinary hospitals. These animals varied in age from 1 to 19 years. The majority were killed because of advanced age. Two had chronic nephritis, one a reticulum-cell sarcoma, and one a postencephalitic syndrome. The first 18 cats were killed by asphyxiation with gas, and the others, by intrapulmonary injection of pentobarbital sodium U. S. P. Of the 36 cats, 23 were female.

The heart, the entire aorta, and the common iliac arteries were removed for study. After fixation in 10% formaldehyde solution U. S. P., the heart and aorta were opened longitudinally, and their gross appearance was noted. Blocks of tissue were then removed regularly from each aorta at the following levels: ascending portion of arch; descending portion of arch; midthoracic region, and lower abdominal region approximately 1.0 cm. above the aortic bifurcation. In several animals, blocks of tissue were removed at other levels where intimal lesions were grossly visible. The aortic sections were em-

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In this paper, the term acid mucopolysaccharide refers to the mucoid ground substance (of the vascular wall) which is stained by the colloidal iron-Prussian blue method.[3] This material also stains metachromatically with polychrome methylene blue and toluidine blue. It is a normal constituent of the aortic wall but is a prominent feature in the arteriosclerotic lesion.

* References 1 and 2.

† References 3 through 5.

No.	Sex and Age in Yr.	Thoracic Aorta					Abdominal Aorta				
		Gross			Microscopic		Gross			Microscopic	
		Plaques	Size, Mm.	Color	Intimal Thickening	Lipids	Plaques	Size, Mm.	Color	Intimal Thickening	Lipids
1	♂	0	....	.....	+	+	0	....	.....	++	0
2	♀	0	....	.....	+	0	0	....	.....	+	0
3	♀	0	....	.....	+	+	0	....	.....	+	0
4	♀	0	....	.....	+	0	0	....	.....	++	0
5	♂	0	....	.....	+	0	1	0.5 × 2	White	+	0
6	♂	0	....	.....	+	0	2	0.5 × 3	White	++	0
7	♀	0	....	.....	++	0	0	....	.....	++	0
8	♀	0	....	.....	+	0	0	....	.....	+	0
9	♀	0	....	.....	+	0	0	....	.....	+	0
10	♀	0	....	.....	+	+	0	....	.....	+	0
11	♀	0	....	.....	++	0	0	....	.....	+	0
12	♀	0	....	.....	+	0	0	....	.....	0	0
13	♀	0	....	.....	+	0	0	....	.....	+	0
14	♀	0	....	.....	+	0	0	....	.....	+	0
15	♀	0	....	.....	+	0	0	....	.....	+	+
16	♀	0	....	.....	+	+	0	....	.....	+	0
17	♀	0	....	.....	+	0	0	....	.....	+	0
18	♀	0	....	.....	+	0	0	....	.....	+	0
19	♂ 5	0	....	.....	+	+	1	0.5	Pale tan	+++	0
20	♀ 19	4	1 × 6 2 1 1	White	++	++	3	1 0.5 0.5	White	++	++
21	♀ 9	1	2.5 × 1.5	White	++	+	0	....	.....	+	0
22	♂ 7	0	....	.....	+	0	0	....	.....	+	0
23	♂ 7	0	....	.....	++	+	0	....	.....	+	0
24	♂ 2½	0	....	.....	+	0	0	....	.....	+	0
25	♂ 4	0	....	.....	+	0	0	....	.....	+	0
26	♀ 6	1	1 × 7	White	++	0	0	....	.....	+	+
27	♂ 5	0	....	.....	+	+	0	....	.....	+	0
28	♂ 15	1	0.5	Pale	+	++	0	....	.....	+	0
29	♂ 12½	0	....	.....	++	0	0	....	.....	++	0
30	♂ 9	0	....	.....	+++	++	0	....	.....	+	0
31	♀ 14	0	....	.....	++	0	0	....	.....	+	0
32	♀ 19	0	....	.....	+	0	1	0.5	Pale	+++	0
33	♀ 1	0	....	.....	+	0	0	....	.....	+	0
34	♀ 8	0	....	.....	+	0	0	....	.....	+	0
35	♀ 13	0	....	.....	+	0	0	....	.....	+	0
36	♂ 14	0	....	.....	++	+	0	....	.....	+	0

bedded and stained as described previously.‡ Multiple blocks of tissue removed from each heart included the anterior descending branch of the left coronary artery, the right coronary artery, the left and right ventricular walls, and the interventricular septum. These blocks were embedded in paraffin and stained with hematoxylin and eosin. Selected sections were treated with a combined Weigert-Van Gieson stain and by the colloidal iron Prussian-blue stain for acid mucopolysaccharides.[7] Frozen sections were made from several cardiac blocks, including major coronary arteries, and stained with Sudan IV and hematoxylin. The microscopic grading of lesions has been described elsewhere.§

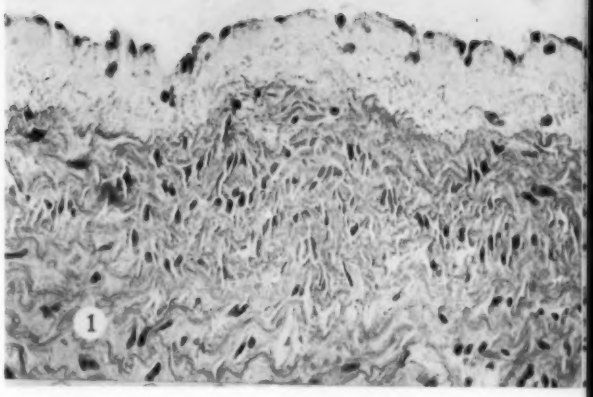


Fig. 1 (Cat 11).—Thoracic aorta showing early mucoid intimal thickening. Hematoxylin-eosin stain; reduced about ⅓ from mag. × 425.

GROSS DESCRIPTION

Thoracic Aorta

Four of the 36 cats had intimal plaques of the thoracic aorta (Table). Single plaques were found in three of the cats. These were white and fibrous, approximately 2 mm. in length, and appeared as ridges in the arch or on the posterior wall of the lower thoracic region. A single, round, intimal nodule about 0.5 mm. in diameter was noted in one animal near an aortic valvular commissure. In the fourth animal, four ridge-like, fibrous, white plaques were observed on the convex surface of the aortic arch near and below its apex. These measured about 1×3 mm. None of the plaques showed evidences of intimal lipid infiltration.

Abdominal Aorta

Intimal plaques were found in the abdominal aorta of 5 of the 36 cats (Table). These were most frequently present on the posterior wall, either between the openings of the celiac axis and the superior mesenteric artery or in the lower abdominal aortic segments. Three animals each showed a single plague; one cat contained two plaques; while another had three plaques above and below the level of the origin of the inferior mesenteric artery. Most of these plaques were slightly elevated, pearly gray in color, and were either circular or elongated and ridge-like. They varied between 0.5 and 3.0 mm. at their widest diameters. One of these abdominal plaques was tan rather than white, but none was yellow, indicating the absence of lipid material.

Heart

Grossly demonstrable lesions of the pericardial and endocardial surfaces, myocardium, valves, and coronary arteries were not observed in any of the 36 cats.

MICROSCOPIC DESCRIPTIONS

Thoracic Aorta

Normally, the endothelium of the thoracic aorta lies directly on the inner layer of the medial elastic tissue, and these structures are separated by only a narrow zone of mucopolysaccharide material. In the medial layer, a thin coating of mucopolysaccharide envelops the individual medial elastic fibers. The earliest lesions observed were characterized by fragmentation, beading, and often by reduplication of the innermost elastic fibers of the medial layer. This degenerative change was accompanied by accumulations of increased amounts of amorphous acid mucopolysaccharide substance,[2] which surrounded the altered segments of elastic tissue and which elevated the adjacent endothelium (Fig. 1). As a rule, the endothelial cells in these areas were swollen and cuboidal, and their cytoplasm contained delicate granules of mucopolysaccharide material. These changes were more pronounced in the arch than in the descending thoracic portion of the aorta, and they were severer on the convex side of the arch and on the posterior wall of the thoracic aorta. Although these early lesions were observed in all 36 cats, they

‡ References 1 through 3.

§ References 1 and 2.

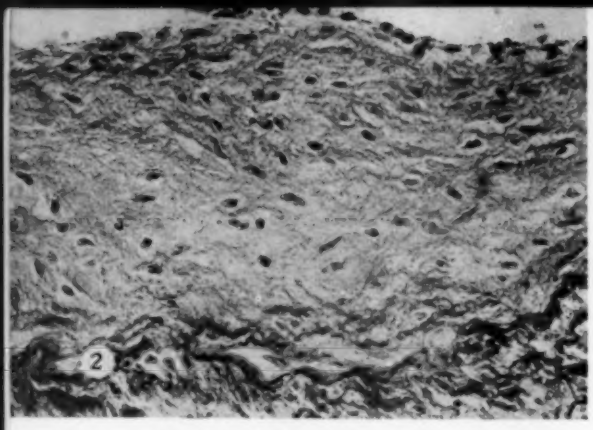
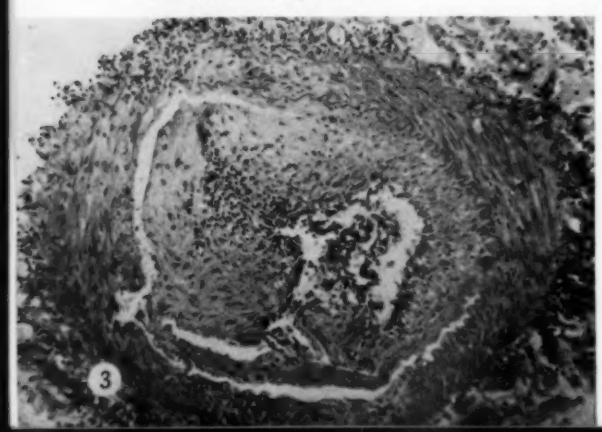


Fig. 2 (Cat 11).—Thoracic aorta showing fibrous intimal plaque. Note fragmentation of inner elastic tissue layer. Weigert-Van Gieson stain; reduced about ⅛ from mag. × 425.

were less pronounced in the younger animals, i. e., the first 18 cats.

As the lesion progressed, either the entire circumference or segments of it showed intimal thickening. The widened intima showed loosening of the subendothelial fibrillary material, and this layer contained scattered small, mononuclear cells and a few circumferentially arranged fibroblasts. These connective tissue cells often had vacuolated cytoplasm containing granular mucopolysaccharide. In most instances the intima was diffusely and uniformly thickened, but in a few cats the intimal process led to formation of discrete intimal plaques. These plaques were usually covered by enlarged endothelial cells and consisted mainly of fibrillary mucopolysaccharide substance (Fig. 2). Fibroblasts were usually more numerous in the superficial portions of these plaques. Both the diffusely thickened intimas and the discrete plaques contained newly formed, delicate elastic and reticulum fibers which were closely associated with the intimal fibroblasts and

Fig. 3 (Cat 22).—Intercostal artery with pronounced fibrous intimal thickening and narrowing of the lumen. Hematoxylin-eosin stain; reduced about ⅓ from mag. × 186.



which were more numerous in the deeper portions of the thickened intima. In several thoracic aortas, the thickened intima was much more compact, was rich in fibrillary mucopolysaccharide, and contained numerous distinct, wavy elastic fibers and coarse reticulum fibers. Collagen was not observed either in the thickened intima or in the intimal plaques. In one cat, the widened intimal layer contained numerous nodular and serpiginous calcific deposits.

The thoracic aortas of 11 of the 36 cats contained small amounts of lipid material. In some instances, the lipid infiltration was limited to a few tiny droplets in the swollen endothelial cells and in the widened, underlying intimal connective tissue. Slightly greater amounts of finely divided sudanophilic substance were observed in the deeper portions of the small intimal plaques. In several thoracic aortas, the inner medial layer contained either a few fine droplets of lipid or linear, streak-like collections of this substance. The lipid stained pale violet with Nile blue in one cat, but the lipid deposits in the other animals did not stain with this material. Examination, with polarized light, of unstained sections of the thoracic aortas of all animals showed a complete absence of refractile material.

Alteration of the medial layer of the thoracic aorta was observed in several animals. Reduplication, condensation, and coarsening of the inner layers of the medial elastic tissue occurred. This was usually accompanied by considerable accumulations of mucopolysaccharide material and, in a few instances, by the appearance of collagenous fibers in the altered inner medial zone. In the deeper layers of the media there were numerous points of disruption of the elastic fibers, and these focal lesions contained increased amounts of mucopolysaccharide substance. The larger medial lesions of this character usually contained a few fibroblasts and dense deposits of collagenous substance. Elongated pools of mucopolysaccharide appeared in the outer portion of the media in one of the cats. The elastic tissue degeneration, mucoid deposi-

tion, and focal fibrosis were more prominent in the thoracic than in the abdominal aorta and were generally severer in the older cats of the second group (19 to 36).

In two cats, intercostal arterial branches of the thoracic aorta showed arteriosclerotic lesions near the origin of each branch (Fig. 3). There was pronounced intimal thickening, with narrowing of the lumens. The intima consisted mainly of fibrillary mucopolysaccharide which, in one plaque, was mildly infiltrated with lymphocytes, monocytes, and a few neutrophilic leucocytes. The endothelial cells over the plaques were enlarged and vacuolated. The internal elastic membrane beneath each plaque was beaded and extensively fragmented. Delicate reticulum fibers were present in each plaque, but elastic tissue and collagenous material were absent. These plaques contained no lipid substance.

Abdominal Aorta

The endothelium is normally separated from the internal elastic membrane by a thin layer of mucopolysaccharide. Thirty-five of the 36 cats showed disease of the abdominal aorta (Table). The lesions observed were more pronounced in the lower abdominal aortic segments and were generally severer on the posterior wall. A process observed in all but one of the animals was focal beading and fragmentation of focal segments of the internal elastic membrane (Fig. 4). In some aortas this process was more diffuse, involving the entire circumference of the abdominal aorta. The degenerating, fragmented segments of internal elastic membrane had lost their refractile quality and appeared granular and often swollen. Vacuoles staining less distinctly were often observed in these degenerating segments. Accumulations of acid mucopolysaccharide were usually found wherever the internal elastic membrane was disrupted. The mucopolysaccharide lay between the internal elastic membrane and the adjacent media elevating the endothelium (Fig. 5). Eventually the degenerated segments disappeared in the mucopolysaccharide substance. In a few instances, small nodules

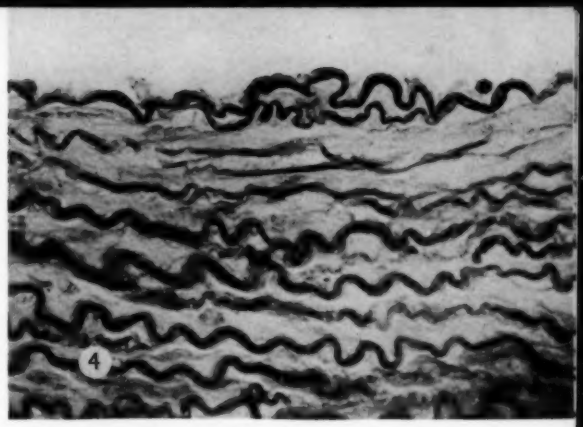


Fig. 4 (Cat 6).—Abdominal aorta showing early degeneration with fragmentation of internal elastic membrane. Note reduplicating elastic layer beneath. Weigert-Van Gieson stain; reduced about ⅕ from mag. × 550.

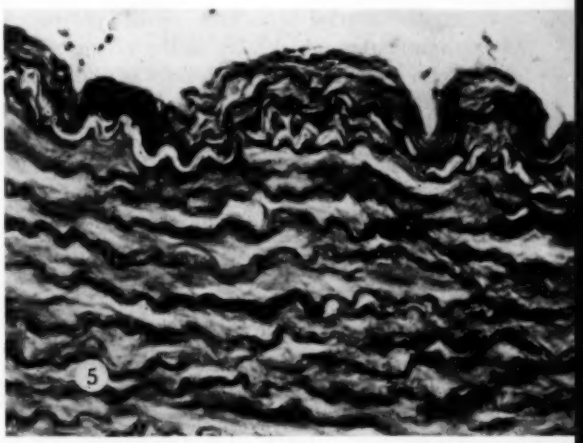
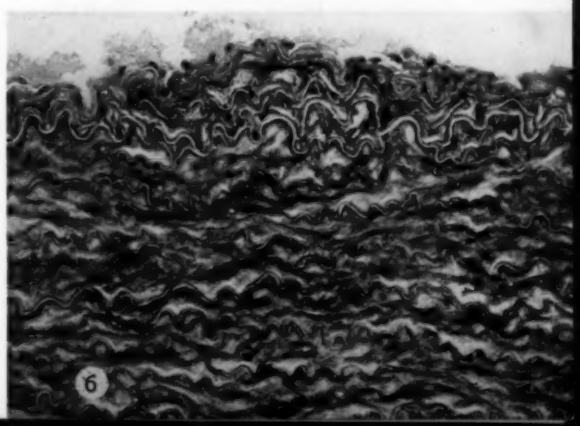


Fig. 5 (Cat 36).—Abdominal aorta showing deposits of acid mucopolysaccharide about degenerating segments of the internal elastic membrane. Colloidal iron-Prussian blue stain; reduced about ⅕ from mag. × 550.

Fig. 6 (Cat 13).—Abdominal aorta with early plaque composed of reduplicated internal elastic membrane and acid mucopolysaccharide. Colloidal iron-Prussian blue stain; reduced about ⅕ from mag. × 425.



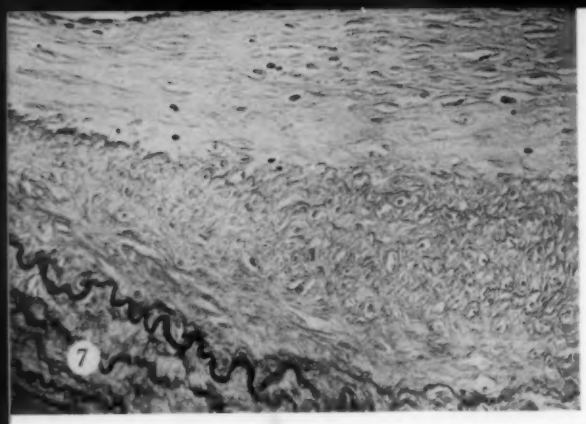


Fig. 7 (Cat 19).—Abdominal aorta showing large intimal plaque consisting of acid mucopolysaccharide and collagen. Note distinct layering of intimal plaque and altered elastic membrane beneath. Weigert-Van Gieson stain; reduced about ⅕ from mag. × 425.

of the medial muscular tissue protruded slightly through defects of the elastic membrane. The defective segments of this membrane were then replaced by intact segments of newly formed elastic tissue appearing in the mucoid ground substance (Fig. 6). The newly formed layer bridged the gap in the membrane, at times leaving residual stubs of the degenerated original segment. Often the regenerative process involved formation of more than one layer of newly formed, internal elastic membrane. The regenerated segments, in turn, appeared to undergo fragmentation in some instances. Where regeneration was more complete, the mucoid deposits were smaller.

In most instances, this degenerative change of the intima led to diffuse intimal thickening characterized by a loosely and irregularly arranged intimal layer composed of fibrillary mucopolysaccharide. Delicate reticulum and elastic fibers appeared in this thickened intima and appeared to be derived from a few scattered, immature fibroblasts within the intimal layer.

In some aortas the intimal thickening was focal and resulted in formation of small intimal plaques. These consisted of irregularly arranged mucopolysaccharide and reticulum fibers. Collagen was absent. Beneath these plaques, moderately severe degenerative changes of the internal elastic membrane were observed. As these plaques enlarged, the mucopolysaccharide, reticulum, and elastic fibers contained within the plaques assumed a circumferential arrangement. The superficial portions of the plaques were more compact, whereas the deeper layers retained mucoid characteristics. Further enlargement of these intimal plaques was associated with increased amounts of reticulum and elastic fibrillary material, and in the largest plaques (Fig. 7) a few collagenous fibers appeared in the deeper layers. In one of these larger plaques a segment of the fragmented, internal elastic membrane partially separated the superficial and deeper layers.

Lipid material was found in lesions of the abdominal aortas of only 6 of the 36 cats. In a few instances the lipid appeared as fine droplets either in vacuolated endothelial cells overlying the thickened intima or along the internal elastic membrane, especially near the fragmented segments and in the adjacent mucoid ground substance (Fig. 8). Fine and coarse droplets were seen in the thickened intima and in the small intimal plaques of three of the animals. In one, no lipid was observed in the plaques, but sudanophilic material was present in linear streaks in the media beneath. The lipid rarely stained with Nile blue, and none showed refractile qualities when observed with polarized light.

Fig. 8 (Cat 28).—Abdominal aorta showing minimal lipid deposition adjacent to fragmented segments of the internal elastic membrane. Sudan IV-hematoxylin stain; reduced about ⅕ from mag. × 425.

CORONARY ARTERIES

Nine of the 36 cats displayed intimal disease of the coronary arteries. In two animals, small coronary arteries had small, eccentrically placed plaques consisting either of acid mucopolysaccharides or of vacuolated fibroblasts containing no lipid material. The me-

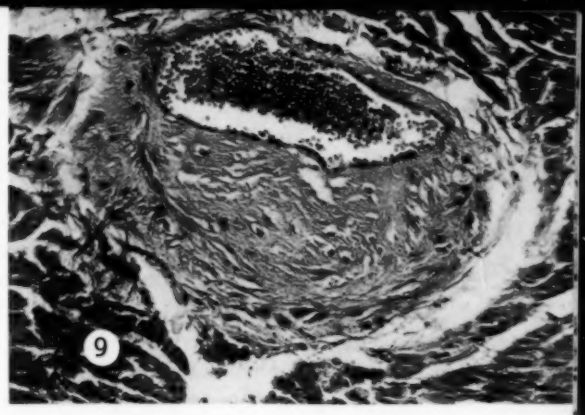


Fig. 9 (Cat 29).—Small coronary artery with eccentric hyaline intimal plaque. Hematoxylin-eosin stain; reduced about ⅙ from mag. × 425.

dium-sized coronary arteries were more frequently involved by disease. The internal elastic membrane of these arteries was fragmented and often reduplicated. The intimal thickening, occasionally eccentric (Fig. 9) but usually concentric, led to narrowing of the vascular lumens. The thickened intima was usually mucoid, consisting mainly of mucopolysaccharide. A few delicate collagenous and reticulum fibers appeared in the thickened intima of only a few vessels. The intimal fibroblasts were occasionally pyknotic, and in one instance had owl-eyed nuclei. Several of these medium-sized arteries had hyperplastic, cellular intimal layers.

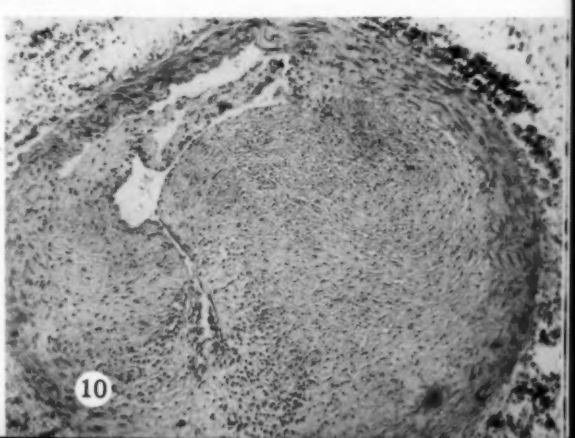
Lesions of the major coronary arteries were observed in only two of the cats. In one, this consisted of multiple points of fragmentation of the internal elastic membrane associated with small deposits of fibrillary mucopolysaccharide. Pronounced alteration of the major coronary arteries of another cat was observed. These lesions were severer in the right coronary artery and in its larger branches than in the left vessel. These vessels showed pronounced intimal fibrous thickening which had led to subtotal occlusion of the lumens (Fig. 10). The intima consisted of loosely arranged, mucoid connective tissue. In the superficial layers of these large plaques, collections of large, vacuolated, connective tissue cells were found. In the smaller and presumably earlier lesions, most of the intima consisted of fibrillary mucopolysaccharides, whereas in the larger, older lesions, greater amounts of reticulum and collagen were seen. However, mucopolysaccharide tended to persist in the midportions in the thickened intimal substance. The internal elastic membrane beneath the thickened intima in these major coronary arteries was flattened, thin, and fragmented. In some vessels the innermost medial elastic tissue was coarsened and reduplicated. The deeper layers of the coronary arterial plaques contained small amounts of lipid material, which appeared as small droplets and which was arranged mainly in circumferential streaks. The lipid was limited to the deeper layers. None of this lipid material was refractile when examined with polarized light.

MYOCARDIUM

Lesions of the myocardium were found in 12 of the 36 cats. In five animals, focal myocardial scars were present, usually in the wall of the left ventricle. In some, atrophic myocardial fibers with pericellular collagenous deposits were seen. Fibrillary mucopolysaccharide was also present in most myocardial scars. One of the larger myocardial scars lay adjacent to several medium-sized coronary arteries, displaying intimal thickening and narrowing of their lumens.

The myocardial lesions in the other seven animals consisted of focal collections of lymphocytes in both ventricular walls and in the interventricular septum. These collections were usually small and, at times, were found near and in the endocardial layer. In one of the older cats, the lymphoid infiltration

Fig. 10 (Cat 22).—Right coronary artery showing extensive intimal thickening with subtotal occlusion of lumen. Note vacuolated macrophages near the endothelial surface of the large plaque. Hematoxylin-eosin stain; reduced about ⅕ from mag. × 186.



was extreme and was found in all cardiac layers. Formation of lymphoid follicles was observed in this lesion; the lymphoid cells were moderately pleomorphic, and large numbers of mitoses were encountered. This lesion suggested a neoplastic process.

COMMENT

This study of the aorta and coronary arteries of the cat suggests that the arteriosclerotic process in this species is initiated by degeneration of elastic tissue. In the thoracic aorta, the initial site of disease was in the innermost medial elastic lamina. In the muscular abdominal aorta, the coronary arteries, and the aortic branches, the earliest lesion was observed in the internal elastic membrane. This degeneration, which is characterized by fragmentation and beading deformity, is severest in the posterior wall and in the inferior portion of the aorta—areas where fully developed arteriosclerotic plaques are most frequently observed. This elastic tissue degeneration is an extremely common process, occurring throughout the aorta, and it is found even in younger animals. The elastic defects appear to heal by formation of a new elastic layer, either in the thoracic or abdominal aorta. All stages of the degenerative and later healing processes are demonstrable. The elastic fragmentation is either accompanied by or followed by accumulations of acid mucopolysaccharide ground substance localized on both sides of the degenerated elastic layer. It would appear that in this feline lesion the newly formed elastic membrane replacing the degenerated segment is derived from the mucoid ground substance. The relation of the mucinous ground substance to elastic fibers has been reviewed by Bunting and Bunting.[8] Since cellular proliferation is not apparent at this stage, intimal fibroblasts cannot be concerned with the development of a new elastic tissue layer. Following regeneration of the elastic membrane, the accumulated mucopolysaccharide is probably resorbed.

Since this degenerative process is common and widespread, and occurs even in young animals, it is obvious that each fracture in the internal elastic membrane is not followed by development of an arteriosclerotic lesion. This study suggests that repeated injuries of the internal elastic membrane occur, possibly by a result of wear and tear from intravascular blood pressure. Nutritional factors may likewise play a part in this early degenerative lesion of the elastic tissue. Since severer changes of this nature are observed in older animals, aging of the vascular tissue may be responsible, in part, for lack of complete elastic tissue regeneration.

At some sites, regeneration of an elastic tissue layer is incomplete or does not occur, and the accumulated mucopolysaccharide is retained in the area. It is possible that repeated injury in the same area may either prevent regeneration or cause further degenerative changes in the newly formed elastic lamina. If the degenerative change is diffuse throughout the circumference of the vessel, the fibrillary mucoid deposits cause a diffuse concentric intimal thickening. Or, if the process is more localized, the mucoid deposits may elevate the endothelium and result in formation of a localized intimal plaque. As the diffusely thickened intima or the intimal plaques become thicker and larger, reticulum and collagenous fibers are formed about the proliferating, intimal connective tissue cells. A few delicate elastic tissue fibers likewise appear in the thickened intima or intimal plaques, and these are derived apparently from the cellular elements of the intima rather than from the preexisting, internal elastic membrane. In the earlier plaques, the cells and fibrillary materials had an irregular arrangement, and, as a plaque enlarged, its components assumed a circumferential arrangement.

It is apparent that lipid material plays no part in the development of the early intimal lesion, either in the diffuse intimal thickening or in the intimal plaque formation. Small amounts of lipid material are found, however, in the slightly thickened intima or intimal plaques, or even at times in the adjacent media, but this lipid infiltration appears to be

a late and secondary process. It is of interest that none of this lipid material was refractile when examined with polarized light, suggesting that no cholesterol was present. Calcific deposits in the thickened intima, associated with lipid infiltration of an intimal plaque, were observed in only one of the animals.

Disease of the media is characterized by focal fragmentation of elastic tissue and is followed by deposition of fibrillary acid mucopolysaccharide and, eventually, by collagenous formation. This medial process is very similar to that characterizing the intimal disease but is much less pronounced. Medial disease is more prominent in the thoracic segment of the aorta, undoubtedly because of the richer supply of elastic fibers.

It is of interest that some of the focal, myocardial fibrotic lesions were closely associated with, and presumably due to, diseased coronary arterial branches. The focal lymphoid collections in the myocardium suggest an inflammatory reaction. In one animal the lymphoid infiltration appeared neoplastic.

The arteriosclerotic lesions found in our cats closely resembled those previously observed in normal dogs [1] and in dogs subjected to thyroidectomy and hypophysectomy.[2] The lesions observed in older dogs,[1] however, were more numerous and were larger than those described here in cats. However, the pathogenesis of lesions in both animals seems to be identical, although in the arteriosclerotic plaques of the aortas of dogs formation of new elastic tissue was much more pronounced than in the feline lesions. Likewise, comparison of the arteriosclerotic lesions of cats with those occurring naturally in the muscular arteries of birds shows that the lesions of the two species are fundamentally similar. The secondary lipid infiltration observed in the lesions of birds, however, is of greater magnitude than that seen in the lesions of dogs and cats.

A recent study of the pathogenesis of arteriosclerosis in human cerebral arteries showed that aging resulted either in diffuse, concentric thickening of the cerebral intimas or localized thickening with plaque formation.[9] In both instances the intima became wider due to deposition of ground substance, fibroblasts, and collagenous and elastic fibrils. The appearance of lipid material in these cerebral vascular lesions was a late occurrence. Early degenerative changes of the internal elastic membrane were believed to be related to the development of the intimal lesion. The pathogenesis of this human cerebral lesion closely parallels that observed in cats in the present study.

SUMMARY

The present report is based on a study of 36 cats, 14 of which were 5 years of age or older.

Naturally occurring arteriosclerosis is common in the cat, particularly in old age. However, it appears to be less severe than in other species at comparable ages and less likely to lead to development of grossly visible intimal plaques.

The initial arterial lesion is characterized by degeneration and fragmentation of the internal elastic membrane or inner medial elastica and is accompanied by deposition of acid mucopolysaccharides. This basic lesion occurs repeatedly and probably often heals by regeneration of elastic tissue. The internal mucoid deposits may, however, persist either diffusely or focally and may lead to the development of a diffusely thickened intima or localized intimal plaques. Collagenous, reticulum, and elastic fibers gradually replace the mucoid ground substance as the plaques mature.

Disease of the aortic media has a similar pathogenesis but is minimal.

Lipids are rarely observed in the early intimal lesions and do not appear to be concerned in their pathogenesis. Cholesterol is not found in older lesions containing other lipid substance.

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News and Comment

PERSONAL

Retirement of Dr. George Hoyt Whipple.—On June 30, Dr. George Hoyt Whipple retired as Professor of Pathology and Head of the Department, at the University of Rochester School of Medicine and Dentistry. Dr. Whipple, Nobel Prize winner in 1934, and one of our most distinguished pathologists, has been Professor of Pathology and Dean of the School of Medicine and Dentistry since 1921.

Appointment of Dr. James L. Orbison.—Dr. James L. Orbison has been appointed as the first George Hoyt Whipple Professor of Pathology and Head of the Department of Pathology, at the University of Rochester School of Medicine and Dentistry. Dr. Orbison, who has been Associate Professor of Pathology, at the Western Reserve University School of Medicine, took over his new post on July 1.

Appointment of Dr. Richard W. Tiecke.—Dr. Richard W. Tiecke, formerly deputy chief of the oral pathology branch, of the Armed Forces Institute of Pathology, Washington, D. C., has been appointed Associate Professor of Dental Pathology in the Northwestern University School of Dentistry.

Appointment of Dr. Ernest W. Goodpasture.—Dr. Ernest W. Goodpasture, of Vanderbilt University, has been appointed Scientific Director of the Department of Pathology, of the Armed Forces Institute of Pathology, Washington, D. C. He assumed this position on July 1 and is responsible for the supervision of the professional activities including diagnostic services in pathology, an advanced teaching program, and experimental studies in pathology and related fields.

On May 9, Dr. Goodpasture was awarded the Howard Taylor Ricketts Medal for 1955 by the University of Chicago. The award was made in recognition of his many years of fruitful research in the field of viral diseases.

DEATHS

Dr. Jacob Werne.—Dr. Jacob Werne, Associate Clinical Professor of Pathology at the New York Medical College, died April 14.

ANNOUNCEMENTS

Annual Meeting of Inter-Society Cytology Council.—The Third Annual Meeting of the Inter-Society Cytology Council will be held at the Statler Hotel, Cleveland, on Friday and Saturday, Nov. 11 and 12. Everyone interested in cytology is invited to attend. Additional information may be obtained from the Office of the Secretary-Treasurer, 634 North Grand Ave., St. Louis 3.

Mucoceles of the Appendix and Peritoneal Pseudomyxoma

CHARLES C. CARLETON, M.D., New Orleans

In 1934 d'Aunoy and Fine [1] reported the single case of peritoneal pseudomyxoma of appendiceal origin which had been observed at the Charity Hospital of Louisiana, at New Orleans, during the previous 27 years. They found only 90 such cases reported in the literature up to that time. Recently, 19 years later, a second case has occurred at Charity Hospital. This second case, together with 13 cases of mucocele of the appendix, is the basis of this inquiry into the relationship between these two conditions.

The original observation of the relationship of a cystic appendix or mucocele to peritoneal pseudomyxoma was made by Fraenkel * in 1901. The term "pseudomyxoma peritonei" had been introduced by Werth † in 1884 to describe a condition in which the peritoneal cavity had become filled with gelatinous material following rupture of a cystadenoma of the ovary. The prefix "pseudo" is meaningless, since it has been pointed out that "pseudomucin" is simply old mucin.[7] Werth considered pseudomyxoma to be a reaction of the peritoneal tissues to the presence of mucus, a type of non-neoplastic foreign-body peritonitis, and postulated a serosal origin of the columnar cells lining some of the cysts. Fraenkel's report of the same condition arising from a ruptured cystic appendix lent support to Werth's theory of an inflammatory-exudative reaction of the peritoneum to mucus. Numerous papers have appeared since, some ‡ supporting Werth's concept, others § challenging it and supporting an implantation or cellular theory. The cellular implantation theory obtained some additional support when the suggestion was advanced that some mucoceles undergo "adenocarcinomatous degeneration."[8] Later Woodruff and McDonald [9] reported 10 cases of cystic appendix in which the epithelia showed papillary formations, which they labeled "adenocarcinoma, Grade 1," and interpreted "as the result of malignant changes which took place somewhere in the pathogenic cycle of the simple mucocele." They suggested that it is only these "malignant mucoceles" which can give rise to peritoneal pseudomyxoma. Several reports have appeared since, in which the "cystic tumors" of the appendix have been set apart as a separate entity distinct from simple mucocele.‖ An analysis of the data from the largest series of these "cystic tumors," published by Hilsabeck and coworkers,[11] fails to show that the mucoceles they described as malignant on histological grounds were actually malignant clinically. Only 3 of their 18 cases of "malignant" mucoceles had ruptured. In the single case in which necropsy findings were recorded, the presence of a pseudomucinous cystadenoma of the ovary casts some doubt on the role of the mucocele. This leaves only two cases in which death might properly be attributed

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From the Department of Pathology, Tulane University of Louisiana, and the Laboratory of Pathology, Charity Hospital.

* Fraenkel, E., cited by Trotter.[2]

† Werth, R., cited by Trotter.[2]

‡ References 2 and 3, and Monod, R., and Vuillième, cited by d'Aunoy and Fine.[1]

§ References 4 through 6.

‖ References 10 and 11.

Table of Mucoceles 1937-1952

Case No.	Surgical or Autopsy No.	Race	Sex	Age	Brief Clinical Note	Pathological Anatomy of the Mucoceles
1	S42-2275	N	M	23	Appendix removed following diagnosis of acute appendicitis; seen 3 mo. later; apparently well	Appendix: 8 cm. in length; distal half dilated and filled with mucus; 2 cm. in diameter; fecolith present proximal to dilated region; mucosa showed dilatation of glands and early papillary formations; acute inflammation
2	S43-3975	N	M	50	Exploratory laparotomy was done following history of R. L. Q. pain intermittent for 2 yr.; worse recently; appendix removed; no follow-up	Appendix: 8 × 0.6 cm.; in the tip there was a region of dilated glands of Lieberkühn just distal to a point of constriction caused by a band of fibrous tissue
3	S49-1260	N	M	22	Appendiceal abscess found at operation; 8 wk. later appendectomy was performed; well 2 wk. after release	Appendix: 7 × 1.5 cm.; filled with mucus, pockets of mucus in the wall; epithelium showed regions of atrophy and regeneration; papillary formations were also present in one region
4	S51-5634	N	F	69	Appendix was removed during an abdominoperineal resection for carcinoma of the anus; 2 mo. after operation she was apparently well	Appendix: 6.5 × 1.3 cm. in distal one-third, proximal two-thirds 0.8 cm. in diameter; mucosa was composed of numerous papillary structures
5	S50-1265	N	M	21	Gangrenous appendix removed at laparotomy following history of R. L. Q. pain for 1 wk.; no follow-up available	Appendix: 7 × 3.5 cm.; gelatinous cast in lumen; acute inflammation and fresh granulation tissue in wall; mucosa showed dilatation of glands and early papillary formation
6	S39-6640	N	F	39	During a hysterectomy for leiomyomata a cystic mass was found in the region of the cecum; this was ruptured on resecting it and mucoid material escaped; it was found to be the appendix; no follow-up available	Appendix: 6 × 3 × 2 cm.; filled with mucus; there were papillary structures in the mucosa
7	S38-1524	W	F	62	Three wk. following an acute episode of cholecystitis the gall bladder and appendix were removed; no follow-up	Appendix: 7 × 1.5 cm.; thin-walled; epithelium is atrophic or absent; at one point there was a pocket of mucus in the wall
8	S41-799	N	F	47	During a hysterectomy for leiomyomata the appendix was noted to be enlarged and removed; 3 yr. later she had no G. I. complaints	Appendix: 6.5 × 2.5 cm.; lumen filled with firm translucent cast; mucosa was flattened for the most part; a few papillary structures were noted
9	S49-12807	N	F	23	Due to certain masculinizing signs, patient was suspected of having an ovarian tumor; biopsy material taken from the ovaries and appendix removed	Appendix: 8 × 0.7 cm. proximal and 1.0 cm. distal half; lined by atrophic epithelium; 3 papillary formations noted; small diverticulum (2 × 2 mm.) present
10	A53-169	W	M	74	After a long illness manifested by weakness, cough, chest pain, weight loss, the patient became comatose and died; an autopsy was performed; extensive bronchogenic carcinoma found	Appendix: 9 × 0.8 cm.; near the base the lumen was closed by fibrous tissue; epithelium was not identified; lumen was filled with mucus
11	S41-6096	N	F	36	An exploratory laparotomy was done with a postoperative diagnosis of appendicitis versus acute salpingitis; pelvic peritonitis found; normal appearing appendix removed; patient died 36 hr. postoperatively; autopsy done; bronchopneumonia cause of death	Appendix: 13 × 0.8 cm.; serosa roughened by fibrous tags; lumen dilated to 0.5 cm.; mucosa showed regions of dilatation of glands
12	S43-6540	N	F	62	During the repair of a ventral hernia the appendix was removed prophylactically; she was well 6 wk. later	Appendix: 10.5 × 0.9 cm.; mucosa showed both dilated glands and papillary formations
13	S47-4082	N	F	45	At laparotomy for uterine leiomyomata the appendix was noted to extend into the cul-de-sac, where it was adherent to a 3 cm. cystic mass; tip of appendix ruptured in removal; well 1 yr. later	Appendix: 9 × 0.6 cm. proximal two-thirds; distal two-thirds 1.5 cm.; at junction lumen obliterated by carcinoid; attached to tip was cyst wall; mucosa contained papillary structures

on clinical grounds to "pseudomyxoma peritonei" arising from a "malignant" mucocele. As these authors admit, most of their "malignant" mucoceles were effectively controlled by simple appendectomy whether they had ruptured or not. This fact speaks for itself.

THE APPENDICEAL MUCOCELE

"Mucocele" is defined as an appendix, a segment of which is dilated and filled with mucus. Twelve surgically removed appendices have been so diagnosed at Charity Hospital during the past 15 years (Table) from well over 10,000 surgically removed appendices examined during that period. This corresponds roughly with the incidence of 0.07% recorded by Warren and Warren [12] in a large series. One mucocele encountered by the author at autopsy is also included. The ages of the patients ranged from 21 to 74. Five were men and eight were women.

In every case there was evidence of some lesion producing either complete or partial obstruction of the lumen of the appendix. In several the total diameter was not increased, but in these the wall was thinned and the lumen correspondingly dilated. Obstruction was caused by a fecalith in one case and by a carcinoid in another. In the others there was fibrosis of the wall with varying degrees of obstruction due to scar tissue.

In none of the 13 cases of mucocele was there any clinical observation or gross or microscopic pathological finding suggestive of malignancy, such as evidence of invasion or metastasis, atypical cells, abnormal or numerous mitotic figures, or disorganized gland formation. In the two surgical cases later coming to autopsy, one died postoperatively of pneumonia, the other of bronchogenic carcinoma. One had a carcinoma of the anus. In some of the other cases follow-up was unsatisfactory.

Three distinct histological changes in the mucosa could be recognized. In some instances two of these changes were present in the same appendix. It is probable that these three histological appearances do not repre-

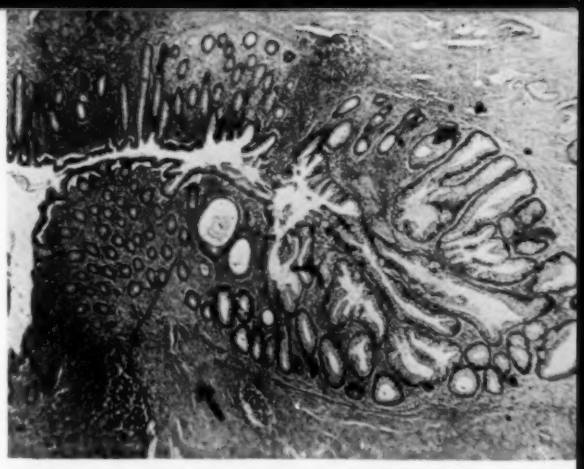


Fig. 1 (Case 2).—Longitudinal section showing localized dilatation of the glands of Lieberkühn in the tip of the appendix. Arrow indicates band of fibrous tissue producing partial obstruction of the lumen; × 30 (slightly enlarged).

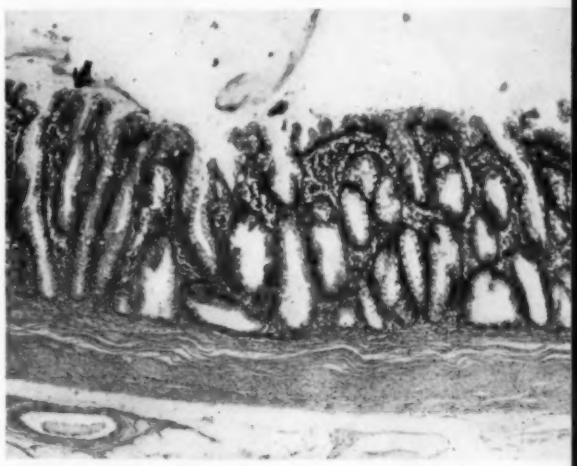


Fig. 2 (Case 11).—Segment of mucosa showing dilated glands. Note the early papillary formation at the left and the absence of lymphoid tissue. Reduced about ⅙ from mag. × 100.

Fig. 3 (Case 12).—Papillary formations of the mucosa lining the mucocele. Note the scanty interglandular and lymphoid tissue. Reduced about ⅕ from mag. × 30.

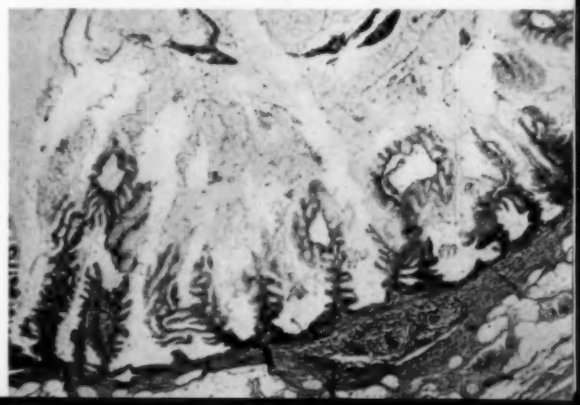


Fig. 4 (Case 4).—Section showing the details of the papillary formations. The nuclei are crowded and somewhat hyperchromatic. The scanty interglandular tissue appears detached from the epithelium in the tip of the papilla. Reduced ⅕ from mag. × 450.

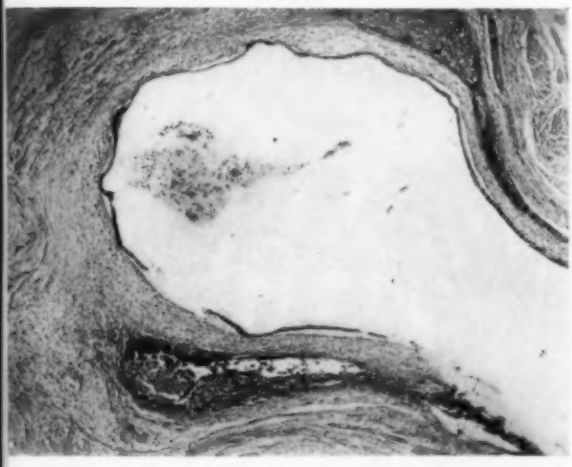
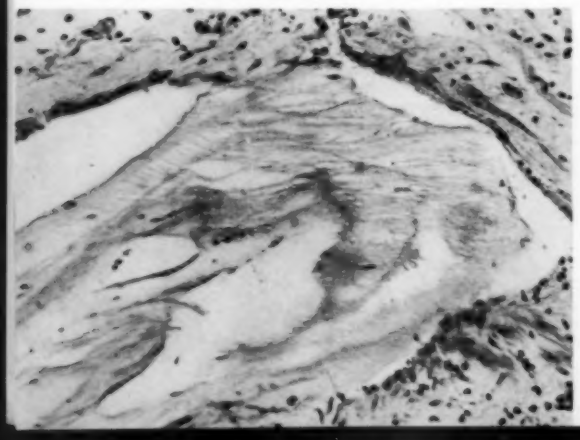


Fig. 5 (Case 9).—Diverticulum of the appendix lined by a low cuboidal epithelium. There are breaks in the mucosa and mucus is present in the lamina propria. Reduced about ⅕ from mag. × 30.

Fig. 6 (Case 3).—From an area of "localized pseudomyxoma" in the wall of a ruptured appendix removed two months after rupture. Note the tendency of the cells at the upper left to form a serosal lining enclosing the pocket of mucus. Reduced about ⅕ from mag. × 450.



sent distinct entities, but only progressive stages in the development of the mucocele.

1. *Glandular Dilatation.*—In five cases regions of dilatation of the glands of Lieberkühn were present. In one (Case 12), a considerable length of the mucosa showed dilatation of the glandular crypts, which had led the pathologist to make a diagnosis of "an adenocarcinoma confined to the epithelium of the appendix." Three cases (Cases 1, 4, and 12), in addition to glandular dilatation, also showed regions of papillary formations characteristic of the second histological picture to be described. This suggests that these appendices represent a transition between Stage 1 and Stage 2.

2. *Papillary Formation.*—In 7 of the 13 appendices papillary structures were observed projecting into the lumen. These were composed of compressed hyperplastic epithelium covering a thin stalk of connective tissue. In some regions the epithelium was apparently detached from the underlying connective tissue (Fig. 4). This may be of importance in the pathogenesis of peritoneal pseudomyxoma. The underlying lymphoid tissue was scanty or absent, and, in one case, the mucosa rested directly on the muscularis with almost complete absence of submucosal lymphoid tissue.

3. *Epithelial Atrophy.*—In five cases the appendiceal lumen was lined by a single layer of flattened or cuboidal epithelium with varying amounts of underlying lymphoid tissue, or by no epithelium at all. In two of these (Cases 8 and 9) a few small papillary structures were also present, suggesting that this stage is related to the papillary stage just described. In some cases there was hypertrophy of the muscularis, while in others it was atrophic. In most of them there was considerable fibrosis of the submucosa.

It has been proposed that the papillary formations found in mucoceles represent a hyperplasia of the epithelium of the appendix or even early adenocarcinoma. However, with the obstruction of the lumen of the appendix, this glandular epithelium is subjected to increased intraluminal pressure, which alone could account for some of the observed

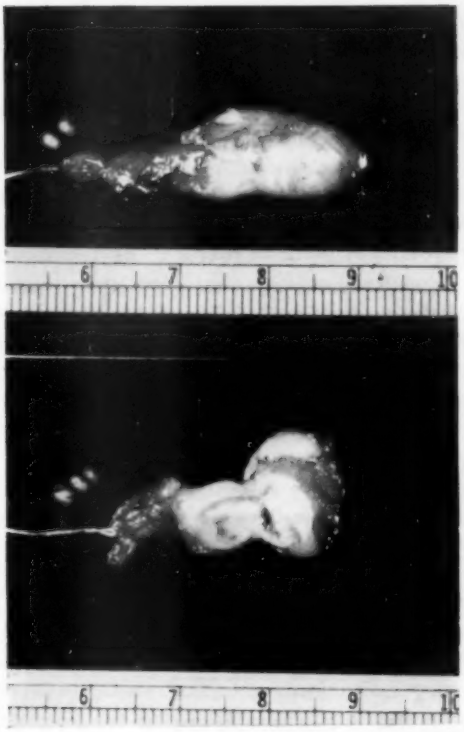


Fig. 7 (Case 14).—Upper: Appendix from the case of generalized pseudomyxoma showing the roughened but intact serosal surface.

Lower: Cut section of the unfixed appendix showing the markedly thickened wall and the slightly dilated lumen.

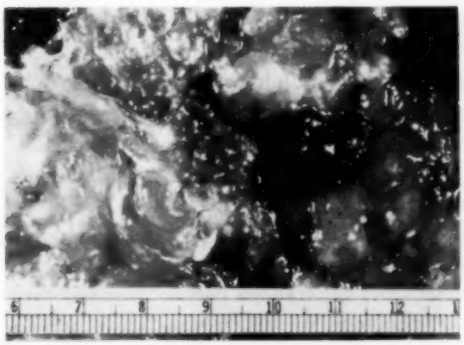


Fig. 8 (Case 14).—Peritoneal pseudomyxoma. Omental tissue showing mucus-containing cysts, some of which are opened.

changes. The sequence of events that follows obstruction of the lumen of the appendix may well be as follows: The increased pressure in the lumen first causes a dilatation of the glands of Lieberkühn. This was the chief

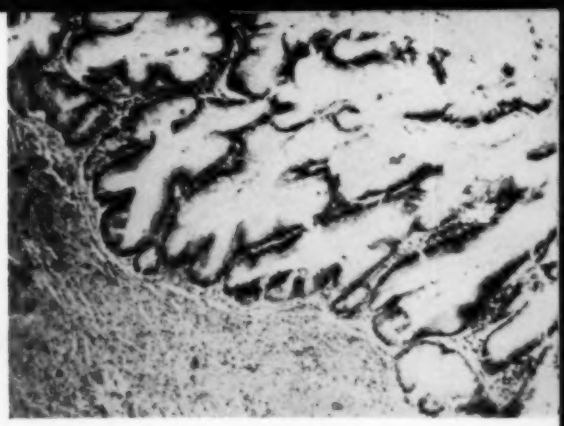


Fig. 9 (Case 14).—Peritoneal pseudomyxoma. Segment of mucosa lining the distal two-thirds of the appendix. Note the greatly dilated glands of Lieberkühn and the absence of underlying lymphoid tissue. The interglandular stroma is reduced to thin strands. The epithelial cells are hyperplastic and hyperchromatic, but there is no evidence of invasion or of cells invading their own stroma. Reduced 1/5 from mag. × 100.

histological change in five of the cases studied. As the pressure increases, the glands are further distended at the expense of the lamina propria and the resulting papillary structures are actually the remains of preexisting interglandular tissue compressed to thin fibrous bands between greatly dilated glands. The rupture of such a mucocele at this time, when it is lined by a hyperplastic epithelium, could possibly lead to the development of a peritoneal pseudomyxoma.

The detachment of the epithelium from the underlying connective tissue and actual breaks in the mucosa could give the cells an increased growth potential and cause them to grow in a way analogous to the cells of the epidermis in repairing a wound in the skin. It appears obvious that rupture of a mucocele, when lined by such a hyperplastic epithelium, would to a certain extent tend to favor the establishment of a fistula between the lumen of the appendix and the peritoneal cavity. Certainly, these hyperplastic epithelial cells would be more apt to line a fistula tract and prevent healing of the rupture than the ones forming an atrophic epithelium.

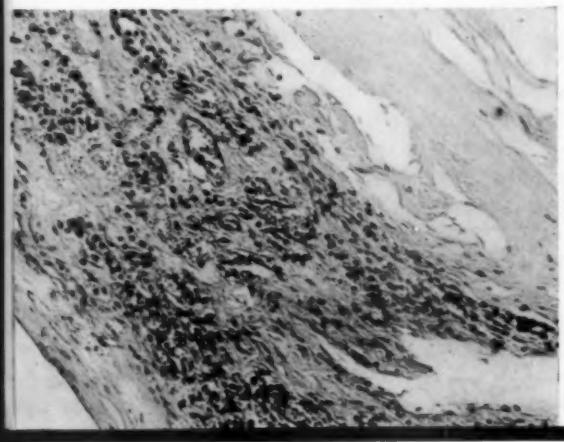
All three histological pictures encountered in mucoceles of the appendix could thus be explained as the direct result of increased intraluminal pressure. In support of this mechanical concept is the fact that similar papillary formations have been described by two independent investigators ¶ in experi-

¶ References 13 and 14.

Fig. 10 (Case 14).—Peritoneal pseudomyxoma. Epithelium lining one of the small mucous cysts in the omental tissue. There is a gradual transition from a flat to a columnar epithelium. Reduced ⅓ from mag. × 450.

Fig. 11 (Case 14).—Peritoneal pseudomyxoma. Papillary epithelium seen in a few of the cysts. Note the dense, almost acellular, fibrous tissue surrounding the cyst. Reduced ⅓ from mag. × 450.

Fig. 12 (Case 14).—Peritoneal pseudomyxoma. Illustration of the highly vascular stroma separating the mucous cysts in the omental tissue. Collections of small round cells are prominent. Reduced ⅓ from mag. × 100.



mental mucoceles produced by ligating the cecal appendage in rabbits. The lack of the usual signs of malignant lesions other than hyperplasia and hyperchromatism make it possible that some of the cases reported as "carcinoma in situ" of the appendix [18] and mucoceles with "malignant epithelia" are actually examples of hyperplastic epithelia produced by pressure effects.

Small mucous cysts were found in three of the cases (Cases 3, 7, and 13) either in the wall of the appendix or in the periappendiceal tissue. The stroma enclosing these cysts of "localized pseudomyxoma" was identical to that seen in the single case of generalized peritoneal pseudomyxoma. This stroma was very vascular, with a generous sprinkling of small round cells with hyperchromatic nuclei, some of which were obvious lymphocytes (Figs. 12 and 13); other cells, while still possessing a hyperchromatic nucleus, assumed a somewhat spindle shape. In Figure 6 these spindle cells can be seen lining up to form a wall around a pocket of mucus. Transition forms can be seen in the surrounding tissue. The mucous cysts varied in size from microscopic up to about 2 cm. in diameter. In sum, the stroma of the tissue separating the pockets or cysts of mucus was characterized by marked vascularity, lymphocytes, and fibrous tissue, all of which are features of granulation tissue. The cells lining some of these cysts appeared to be derived from the reacting stromal cells (Fig. 6).

Actually, these cases of localized pseudomyxoma are equally important as the more spectacular generalized cases. If it could be determined why these mucoceles did not produce a generalized pseudomyxoma, then the problem would be practically solved. Certain points appear suggestive. Two of these appendices had atrophic epithelium, with only the occasional papillary formation. In the third case (Case 13), while papillary formations were fairly numerous, they were very low and the epithelium appeared atrophic. In none was there vigorous hyperplastic epithelium such as seen in Case 4

(Fig. 4) or in the appendix from our case of generalized pseudomyxoma (Fig. 12).

GENERALIZED PERITONEAL PSEUDOMYXOMA

As d'Aunoy and Fine emphasized, the term peritoneal pseudomyxoma describes a syndrome, not a disease. The syndrome has been reported as a consequence of several conditions in which mucus is discharged into the peritoneal cavity, such as ruptured peritoneal cysts, mucoceles, and ovarian cysts. The case of peritoneal pseudomyxoma presented here is fairly typical.

REPORT OF CASE

CASE 14 (J. T.; T-52-83160).—This patient, a 24-year-old white man, was well until about two years prior to the present admission, when he first noticed abdominal swelling. He consulted several physicians. He had three operations and received a course of x-ray therapy without any noticeable benefit. When discharged, his abdomen measured 38 in. (96.52 cm.) in circumference, and little change was noted during the subsequent year. He was referred to Charity Hospital for further treatment a year after his second operation. The patient was asymptomatic on admission except for postprandial dyspnea, vague abdominal discomfort, and the mechanical inconvenience of a greatly distended abdomen.

Past History.—At age 15 he had what was diagnosed as appendicitis, which was treated conservatively with ice bags. He had been short of breath for about two years. He had had hemorrhoids for one year.

Physical Examination.—Temperature, 99.0; pulse rate, 92; blood pressure, 122/80; respirations, 15.

The patient was a well-developed white man with a pale complexion, a tremendously distended abdomen, and somewhat wasted extremities. He appeared chronically ill but was in no obvious distress. Pertinent physical findings were limited to the abdominal region. The abdomen was protuberant and had produced a widening of the costal angle. The upper half of the abdomen was firm to palpation, while the lower half was soft. No definite fluid wave was demonstrated. Bowel sounds were heard. Two surgical scars were noted. On the right there was a large scrotal mass which could not be reduced. On rectal examination a bulging cul-de-sac could be felt.

In the hospital gastrointestinal x-rays revealed no abnormality. Proctoscopic examination was negative. The results of all laboratory tests were within normal limits except for a slight anemia.

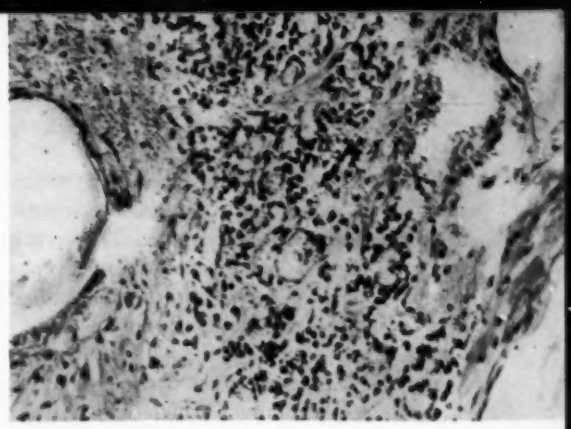


Fig. 13 (Case 3).—Note the similarity of the stroma reaction in this case of "localized pseudomyxoma" to the response seen above (Case 14) from a case of generalized pseudomyxoma. Reduced about ⅓ from mag. × 100.

Course.—Two weeks after admission a laparotomy was performed, and 6 liters of jelly-like mucoid material were removed, together with the appendix and part of the omentum. Three weeks later he was discharged.

He was seen one month later and said he was feeling much better and that his abdomen had decreased even further in size since leaving the hospital. He failed to return for his second clinic appointment, but he was reported doing farm work one year later. No recent follow-up is available.

Pathological Report (S-52-11862).—The specimen consisted of a mat of thick, reddish-gray tissue, embedded in which were numerous small thin-walled cysts. The specimen measured 21 × 16 × 4 cm. and was triangular in general outline. The cysts varied from 0.3 to 3.0 cm. in diameter and were filled with a glairy, stringy, mucoid material. Also included was a fragment of similar tissue, embedded in which was the appendix which, when dissected out, measured 3.5 cm. in length and 1.0 cm. in diameter at the tip. The cut section showed the wall to be thickened and the lumen filled with translucent mucus.

Microscopically, the appendiceal wall was thickened, principally by increased fibrous tissue, but there was, as well, some slight hypertrophy of the muscularis. The lumen was 0.3 cm. in its greatest diameter and contained homogeneous pink-staining stringy material. The crypts of Lieberkühn were dilated, and papillary formations were present in the distal thickened portion similar to those described earlier in the other mucoceles. In the proximal region near the base of the appendix, the lumen was narrowed to a pinpoint opening by scar tissue. The mucosa proximal to this was normal.

The microscopic appearance of the tissue removed at laparotomy was characteristic of peritoneal pseudomyxoma. The small mucus-filled cysts were separated by granulation tissue and dense fibrous tissue. The granulation tissue was quite vascular and contained small collections of small round cells. The cysts were lined either by no cells at all or by a cellular lining, which varied from a flattened endothelial type to a cuboidal or columnar type (Fig. 10). In some cysts the columnar cell lining was thrown up in papillary formations, which were surrounded by dense, almost acellular fibrous tissue, suggesting that the papillary formations may have been secondary to a contraction of this scar tissue (Fig. 11).

While the appendix in this case was not a mucocele in the strict sense because the lumen was only slightly dilated, the history of an episode of appendicitis some years earlier, which was treated conservatively, makes it probable that the appendix was the source of the peritoneal pseudomyxoma. Further, its lumen was partially obstructed, it was filled with mucus, and the epithelium was similar to that seen in other mucoceles. No point of rupture or fistula could be demonstrated in the appendix, but the patient had had two previous operations, the second one involving exploration of the cecal region, following which an opening in the appendix could have been closed by scar tissue. The fact that the size of the abdomen has remained stationary for over a year after his second operation suggests that such was actually the case.

COMMENT

A fundamental problem in the pathogenesis of peritoneal pseudomyxoma has been the origin of the columnar cells lining some of the mucous cysts in the peritoneal tissues.

Some observers have been impressed by the resemblance of these cells to the epithelium which lines the appendix, so much so that they assumed the appendix to be the origin of these cells and that these cells or their progeny produce the mucus which characterizes the condition. This view is put forward in some textbooks of pathology (Boyd,[16] Anderson [17]) and is probably the one most widely held today.

The fact that most cases fail to progress once the mucocele is removed points to the appendix as the source of the mucus. This is in contrast to those cases of peritoneal pseudomyxoma arising from a ruptured ovarian cyst. Here the condition may continue to progress following removal of the cyst; or else it may be initiated by rupture at the time of removal. Some of these women have to have repeated operations for the removal of the mucus. The high percentage of cases of this admittedly rare condition which have been found incidentally at autopsy suggests that the process may have been static for some time prior to death.

Is the resemblance between the mucosal cells of the appendix and those lining some of the mucous cysts in the peritoneum merely coincidental? It has been shown by von Brunn * and Cunningham [18] that such cells can arise from the peritoneum in response to mild irritants, and transitions from flattened mesothelial cells to tall columnar cells were seen in our case of peritoneal pseudomyxoma, sometimes in the same cyst. The recent reports by the two independent investigators referred to above, in which mucoceles were produced in the cecal appendage in rabbits, favor a serosal origin for these cells and support the concept that peritoneal pseudomyxoma is a type of response to irritation or foreign-body peritonitis. The generally accepted concept that it is produced by epithelial implants to the peritoneum was not substantiated by their work. In Cheng's experiments the appendiceal implants failed to produce mucus and were walled off by fibrous tissue. In an earlier report Grodinsky and Rubnitz [19] had contended that the implantation of epithelial cells was the essential feature in pseudomyxoma; but later, after further experiments, Rubnitz became convinced that some of the results "could not be ascribed to cells and that the animals' tissue response to irritants was responsible for some of the

* von Brunn, M., cited by Cheng.[14]

'implants,' " and he cautioned against quoting the earlier article to substantiate the implantation theory.

Cheng points out that if one adheres to the noncellular inflammatory theory, then one must assume that a fistula between the appendix and the peritoneal cavity is established and maintained over a long period of time to allow for the accumulation of the vast amounts of mucus seen in many cases. While this would be unusual, the condition itself is extremely rare, and the establishment of such a fistula may well be the factor determining which mucoceles will produce peritoneal pseudomyxoma. The case reported by Monod and Vuillième supports this theory. In their case, the appendix was distended with mucus and a small opening was present near the tip, through which mucus was being extruded.

The role of diverticula of the appendix in the establishment of a fistula was emphasized by Gardham and co-workers.[3] While this may play a role in some cases, nothing suggesting a diverticulum was present in the case of generalized pseudomyxoma reported here. However, there was mucus present in the lamina propria of the single example of diverticulum (Fig. 5) encountered in the 13 mucoceles examined in this study.

It has been the occasional case[6] which progresses after the apparently offending appendix is removed which has led to the belief that pseudomyxoma is a form of malignant tumor. Alternative explanations of this type of case are: First, it may well be that the appendix is not the primary source at all. Following rupture of an ovarian cyst or retroperitoneal cyst, the appendix is often secondarily involved and the reaction may cause obstruction to the lumen of the appendix and the formation of a mucocele. It is obvious that the removal of such an appendix would not terminate the process. Second, in advanced cases, such as the one reported here, it is physically impossible to remove all of the mucus. The mucus left behind might act as a mild irritant, causing an exudative reaction. Thus, a certain amount of reaccumulation of fluid might be expected in the advanced cases.

The remaining possibility is that there is a metaplasia of the serosal cells of the peritoneum to mucus-producing cells. Morphologically, a transition from a flat serosal-type lining to a mucous-containing columnar epithelium can be observed (Fig. 10). Whether these cells produced the mucus they contain or simply imbibed it remains to be determined.

SUMMARY

From a morphologic study of 13 cases of mucoceles and a single case of peritoneal pseudomyxoma of appendiceal origin, the development of an appendiceal mucocele could be divided into three stages according to the type of epithelium lining it, and, while at one stage the epithelium is hyperplastic, there is nothing to suggest true malignancy. A transition from a flat serosal-type lining to a mucus-containing columnar epithelium could be traced in some of the pockets of encysted mucus in the peritoneal tissues.

It is my belief that the development of a generalized pseudomyxoma depends upon the rupture of mucocele at a time when it is lined by a hyperplastic mucus-producing epithelium and that a fistula between the lumen of the appendix and the peritoneal cavity must be established. If this fistula heals or the appendix is removed, the condition is almost always arrested. The columnar cells seen in some of the peritoneal mucus collections are probably derived from the peritoneal tissue as a response to mild irritation and are not necessarily mucus-producing.

It is obvious from what has been said that the pathogenesis of this condition is not clear and probably will remain so until it is reproduced experimentally.

Drs. C. E. Dunlap, S. C. Sommers, and S. Warren gave advice in the preparation of this paper.

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News and Comment

PERSONAL

Appointments.—Dr. Robert S. Jason, Professor of Pathology at Howard University College of Medicine, Washington, D. C., has recently been appointed Dean.

Dr. Russell von Milliser has recently been made Professor of Pathology in the Chicago Medical School.

GENERAL NEWS

English and Spanish Summaries of German Articles.—The *Deutsche medizinische Wochenschrift* is now printing English and Spanish summaries of all original articles published in each issue. It is hoped that this will stimulate, among physicians in other countries who know little German, a livelier exchange of medical information with their German colleagues.

ANNOUNCEMENTS

International Symposium on Enzymes.—There will be an International Symposium on Enzymes: Units of Biological Structure and Function, in the auditorium of the Henry Ford Hospital, Detroit, on Nov. 1-3. Many of the foremost investigators in this field will participate. All interested in attending this Symposium should address inquiries to Dr. Clarence E. Rupe, Secretary, International Symposium on Enzymes, Henry Ford Hospital, Detroit 2.

Abnormal Endometrial Changes Induced in the Rat

The Effects of Chorionic Hormone and Estrogen

JAVIER ARIAS-STELLA, M.D., Lima, Peru

Focal atypical endometrial changes characterized by nuclear enlargement of isolated epithelial cells were recently described in certain cases of uterine abortion, ectopic pregnancy, hydatidiform mole, chorioadenoma destruens, and chorioepithelioma.[1] These changes occurred in glands having either proliferative or secretory activity, or both, in varying degrees. The presence of viable chorionic tissue together with the histiological patterns of the lesions led to the postulation that chorionic hormones and estrogens might be the factors concerned in the pathogenesis of these changes. The experiments to be described were undertaken in an attempt to test such an assumption.

It has long been recognized that estrogens exert a stimulating effect on the uterine epithelium. When large or prolonged doses of these hormones are administered to normal or castrated animals of several species, a pattern simulating the cystic hyperplasia of the endometrium, as seen in human beings, can be obtained.* Epidermization of the uterine mucosa is another change which accompanies the use of large doses or protracted administration of estrogens.† The action of the chorionic hormones on the ovaries ‡ and their influence on the weight of the uterus [11] have been well studied. However, not as thoroughly investigated are the endometrial changes induced by chorionic hormones. Squamous metaplasia of the uterine epithelium in normal rats injected daily over a long period of time with a preparation of chorionic gonadotropin has been described.[12] Laqueur and Fluhmann [13] administered chorionic hormones to young female rats previously treated with testosterone and observed a tree-branched mucosal proliferation which they described as "unique."

In the present experiments, normal and castrated animals were given estrogen, chorionic hormones, or both in order to determine the individual and combined actions of these hormones. The results indicate that an abnormal endometrial pattern characterized by unusual secretory activity associated with marked proliferative change can be elicited in the normal rat by the simultaneous influence of estrogen and chorionic hormones. The nuclei of the proliferated epithelial cells tend to show a more vesicular and swollen appearance than normal and occasionally display an unusually large size. These changes do not occur when animals receive the same dosage of estrogen or chorionic hormone alone but can be obtained when castrated rats are injected with large doses of estrogen and progesterone.

MATERIAL AND METHOD

White female rats, 3 to 5 months old, of the Sherman strain were used in all of the experiments. Chorionic gonadotropin (Antuitrin-S, Parke, Davis & Company) was the chorionic hormone employed. Crystalline estradiol dipropionate (Progynon-DP,

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From the Cytochemistry Section, Pathology Division, Sloan-Kettering Institute for Cancer Research, and the Pathology Laboratories, Memorial Center for Cancer and Allied Diseases, New York.

* References 2 through 4.

† References 5 through 7.

‡ References 8 through 10.

Summary of Gross and Histological Findings and Number of Observations by Groups

Groups	No. of Animals	Treatment *	Ovaries, Size	Ovaries, Corpora Lutea	Uterus, Size	Uterus, Glandular Hyperplasia	Uterus, Perinuclear Epithelial Vacuolation	Uterus, Vacuolation with Formation of Large Epithelial Retention Cysts	Uterus, Nuclear Enlargement of Epithelial Cells	Uterus, Focal Areas of Squamous Metaplasia	Uterus, Leucocytic Infiltration in the Mucosa	Uterus, Stroma	Uterus, Myometrial Hypertrophy	Cervix-Vagina Keratinization	Cervix-Vagina Epithelial Hyperplasia	Cervix-Vagina Mucification
I	12	Ch + E	Enlarged 3 to 5 times normal	Numerous, practically replacing the entire parenchyma	Moderately enlarged	Marked, with slight cystic tendency	Marked	Marked	Occasional cells	No	No	Fibrotic and thickened	Yes	No	Yes	Yes
II	10	Ch	Enlarged 2 to 4 times normal	Numerous, practically replacing the entire parenchyma	Slightly enlarged	Slight	No	No	No (see text)	No	No	Fibrotic and thickened	Yes	No	No	Yes
III	6	E	Smaller than normal	Few	Markedly distended up to 6 times normal	Moderate, with cystic tendency	Marked	No	No	Seen in 2 animals	Yes	Fibrotic	No	No (see text)	Yes	Yes
IV	6	E Oophorectomy	.....	.....	Moderate luminal dilatation	Marked, with much cystic change	Marked	No	No	Seen in 2 animals	Yes	Fibrotic	No	Yes	Yes	No
V	4	Ch Oophorectomy	.....	.....	Atrophic	No	No	No	No	No	No	Dense and fibrotic	No	No	No	No
VI	6	Ch + E Oophorectomy	.....	.....	Moderate luminal dilatation	Moderate, with slight cystic tendency	Slight	No	No	Seen in 3 animals	Yes	Fibrotic	No	Yes	Yes	No
VII	6	P + E Oophorectomy	.....	.....	Moderately enlarged	Marked, with slight cystic tendency	Marked	Marked	Occasional cells	No	No	Fibrotic and thickened	Yes	No	Yes	Yes

* Ch indicates chorionic hormone; E, estrogen; P, progesterone.

Schering Corporation) was the estrogen given. The chorionic hormone was injected subcutaneously in a dosage of 100 I. U. daily. The estradiol propionate in oil solution was injected intramuscularly in a dosage of 35γ daily. The period of treatment was 18 to 20 days. Throughout the experiment the animals were maintained on identical dietary (Purina Chow and water ad libitum) and environmental conditions.

The animals were divided into the following groups:

Group I: Normal animals receiving chorionic hormone and estrogen

Group II: Normal animals receiving chorionic hormone alone

Group III: Normal animals receiving estrogen alone

Group IV: Castrated animals receiving estrogen alone

Group V: Castrated animals receiving chorionic hormone alone

Group VI: Castrated animals receiving chorionic hormone and estrogen

Group VII: Castrated animals receiving progesterone and estrogen.

The progesterone used was Proluton (Schering Corporation) and was injected in a dose of 2 mg. daily. This last group received 52.5γ of estrogen daily.

In all the castrated animals injections were begun one day after operation.

Immature and mature untreated animals, at different phases of the endometrial cycle and during lactation, were also studied for comparison.

Vaginal smears stained by the Papanicolaou procedure were made at the beginning and at the end of the treatment period. After completion of treatment the animals were killed with ether anesthesia, and the gross appearance of the uterus, tubes, vagina, ovaries (in the noncastrated groups), adrenals, pituitary, mammary glands, and other organs was recorded. The entire uterus and a representative portion of the vagina were fixed *in toto* for 24 hours and then cut and embedded in paraffin. This procedure yielded more satisfactory histological specimens than cutting the tissues before fixation. Sections were stained with hematoxylin-eosin, Mayer's mucicarmine, and periodic acid-Schiff reagent prior to and after diastase digestion. Histological and histochemical studies of adrenal and pituitary glands were also carried out, but they are the matter of a separate study.

RESULTS

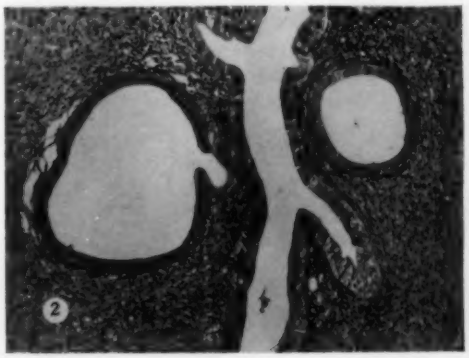
The accompanying table summarizes the gross and histological findings and the number of observations in all the groups.

Fig. 1 (Group III).—Normal animal receiving estrogen. Glandular hyperplasia with moderate cystic tendency. Discrete perinuclear vacuolation. Hematoxylin and eosin; reduced ¼ from mag. × 75.

Group I. Normal Animals Receiving Chorionic Hormone and Estrogen

The ovaries were considerably enlarged in these animals, ranging from three to five times the normal size. Microscopic study showed the parenchyma filled entirely with corpora lutea and scattered follicles undergoing luteinization. The uteri were moderately enlarged, but the lumina were not dilated. The uterine walls were thicker than normal as a result of both mucosal and muscular hypertrophy. The endometrial pattern was one of atypical hyperplasia in which proliferative and secretory changes occurred simultaneously. The surface epithelium showed cylindrical cells with pink cytoplasm and vesicular nuclei. The cells were lined in

Fig. 2 (Group IV).—Castrated animal receiving estrogen. Cystic glandular hyperplasia. Slight vacuolation of the epithelium in focal areas. Hematoxylin and eosin; reduced ¼ from mag. × 75.



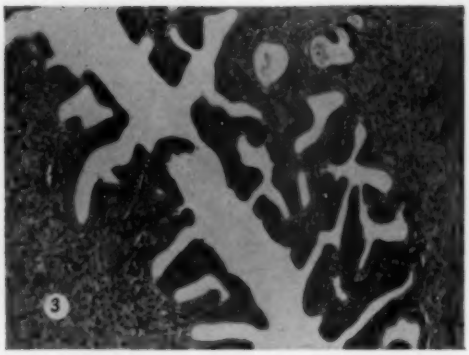
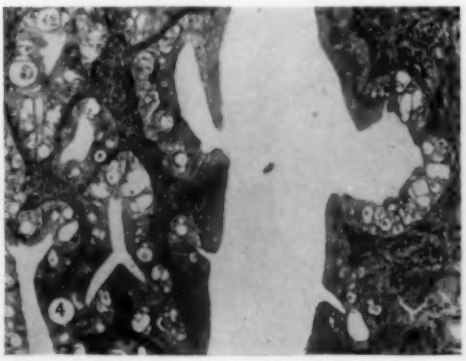


Fig. 3 (Group II).—Normal animal receiving chorionic hormone. Progestational type of endometrium. Observe typical lace-like folding of the mucosa and absence of glandular hyperplasia and epithelial vacuolation. Secreted material is seen in the lumen of two glands near the right upper corner. Hematoxylin and eosin; reduced ¼ from mag. × 75.

one row, but in some segments there was marked proliferation with formation of cell aggregates which extended in the stroma or bulged into the lumen in papillary form. In these areas of proliferation, as well as in other portions of the surface epithelium, there was marked vacuolation. This process was observed starting as perinuclear halos or discrete protoplasmic vacuoles which, by enlarging, could involve the whole cytoplasm resulting in secondary distortion of the nucleus. Frequently the vacuoles acquired an enormous size and by fusion formed large intraepithelial cysts (Figs. 4 and 5). Most of

Fig. 4 (Group 1).—Normal animal receiving chorionic hormone and estrogen. Marked epithelial vacuolation with presence of confluent "retention cysts." Glandular hyperplasia without cystic tendency. Hematoxylin and eosin; reduced ¼ from mag. × 75.



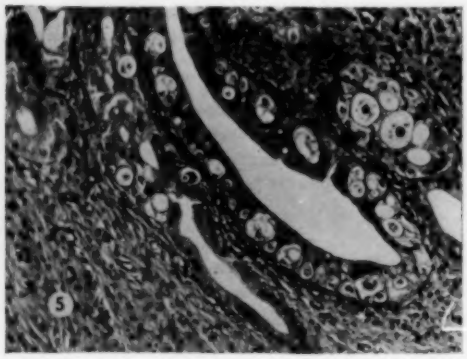
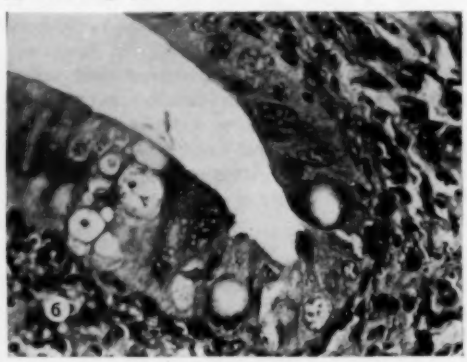


Fig. 5 (Group I).—Epithelial vacuolation, cellular proliferation, nuclear distortion, and glandular involvement are demonstrated. Hematoxylin and eosin; reduced ¼ from mag. × 108.

these cysts appeared in hematoxylin and eosin sections as clear spaces, but in many of them a pink or hyaline central mass was present. These masses gave a positive stain with the periodic acid-Schiff reagent and mucicarmine, and no change in their staining properties was noted after treatment with diastase. In the epithelial cell aggregates, mitosis was occasionally seen. The glands were hyperplastic and showed scattered dilated lumina. The process of vacuolation involved the neck of the glands and all the more superficially situated glands (Figs. 5 and 7). Here the same cytoplasmic change and active proliferation of cells was noticeable. In several animals, the intensity of the glandular vacuolation had an inverse relationship to the degree of glandular hyperplasia. Through-

Fig. 6 (Group I).—High-power view of surface epithelium showing nuclear enlargement, mitosis, and vacuolation. Hematoxylin and eosin; reduced ¼ from mag. × 296.



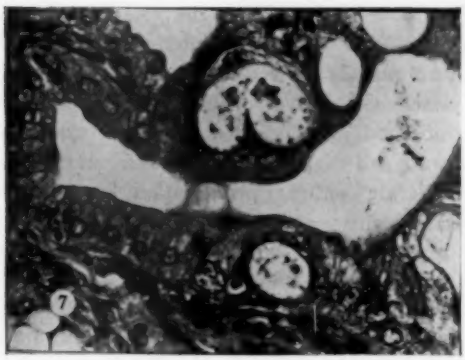


Fig. 7 (Group I).—Gland showing large confluent "retention cysts" and single hypertrophic nucleus (arrow). Hematoxylin and eosin; reduced ¼ from mag. × 360.

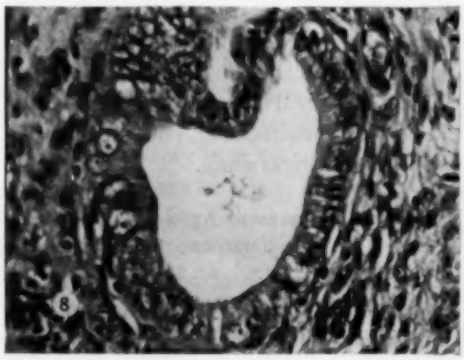
out the areas showing proliferative and secretory changes the nuclei were moderately enlarged, pale, and vesicular and showed a distinct nuclear membrane with a chromatin of swollen appearance (Fig. 6). The nuclear shape was not only distorted by compression due to the vacuoles but also as a result of the disorderly apposition of cells. The loss of cellular polarity was characteristic of the proliferated areas. Occasionally some animals showed cells on the surface or glandular epithelium in which marked nuclear enlargement was present (Figs. 7 and 8). These enlarged nuclei showed an increase in size two or more times that of their neighbors, and this striking disparity was evident even at low magnifications. The stroma was fibrous and congested, and the myometrium, hypertrophic. The cervix and the vagina showed marked epithelial proliferation and mucification, but no keratinization. Here a diffuse and intensive staining with PAS and Mayer's carmine was observed as a result of the mucification, and only a few isolated empty vacuoles were present. Comparing the results of the PAS and Mayer's carmine staining in the endometrium and cervix, it seems apparent that the vacuoles in the endometrium contained a more soluble material in the histological reagents used than the cervical mucin.

One observation made in these animals, which also applies to all other groups, is that the degree of epithelial stimulation in the cervix varied in its different segments. In general the most external portion of the cervix showed less hyperplastic phenomena than the endocervical segment.

Group II. Normal Animals Receiving Chorionic Hormone

The ovaries were enlarged two to four times the normal size and histologically showed replacement of the parenchyma by numerous corpora lutea and a few follicles undergoing luteinization. Occasional cysts lined by a single layer of luteal or follicular cells, or only by connective tissue, were also present. The uteri were slightly enlarged, but the thickness of the walls was significantly increased, mainly due to hypertrophy of the mucosa. The endometrial reaction was that seen in the progestational state (Fig. 3). There were numerous lace-like projections of the mucosa into the lumen, but no glandular hyperplasia. Minimal vacuolation of the epithelium occurred in isolated cells. It was noted that pink or hyaline masses of secreted material filled the lumen of numerous glands. These masses were positively stained with mucicarmine and the Schiff reagent, and no change in the staining property was noted after diastase digestion. Only in one case the epithelial vacuolation approached that seen in the previous group. In this same animal a few enlarged nuclei were noted. The stroma was slightly cellular, fibrotic, and congested.

Fig. 8 (Group I).—Gland showing group of large vesicular nuclei. Hematoxylin and eosin; reduced ¼ from mag. × 385.



The myometrium was hypertrophied to a small extent. The cervix and vagina showed marked mucification and proliferation of cells in several layers but no epidermization or keratinization.

Group III. Normal Animals Receiving Estrogen

The ovaries were somewhat smaller than normal but showed a few corpora lutea and follicles. In one animal the ovaries were completely atrophic. The uteri were markedly enlarged and, in some, dilated up to five or six times the normal size. The lumen was filled with dense leucocytic exudate. In the dilated portions the mucosa was very thin and limited to one row of cubic or flattened cells, with the stroma and myometrium equally compressed. In the less dilated segments there were frank endometrial hyperplasia. Folds of mucosa projecting into the lumen and glandular proliferation with dilatation and cystic tendency were present (Fig. 1). In some areas the surface and glandular epithelium showed marked perinuclear vacuolation accompanied by secondary shrinkage of the nuclei, only rarely forming large vacuoles. Confluent cytoplasmic cysts, similar to those described in Group I, were not present. Cellular proliferation of the surface epithelium occurred in isolated areas. In this group marked leucocytic infiltration, leading in some cases to ulceration, was observed in the mucosa. Two animals showed squamous metaplasia at the proximal end of the corpus uteri in areas presenting marked vacuolation and leucocytic infiltration. In the less distended segments the myometrium was slightly hyperplastic. In the cervix and the vagina there was moderate mucification of the upper layers and proliferation of the basal cells. The animal showing atrophic ovaries did not show mucification, but keratinization and epithelial hyperplasia.

Group IV. Castrated Animals Receiving Estrogen

The uteri were dilated and moderately enlarged. Histologically, the changes in these animals were similar to those in the previous group, except that here the glandular cystic hyperplasia was more intensive (Fig. 2). Two animals showed foci of squamous metaplasia. The myometrium was moderately thickened in nondilated segments, and the cervix and vagina showed keratinization with moderate epithelial proliferation, but no mucification.

Group V. Castrated Animals Receiving Chorionic Hormone

At the dosage given the chorionic hormone seemed to have no significant effect on the process of involution of the uterus following castration. Marked uterine atrophy was uniformly seen in all cases. Histological examination showed a surface and glandular epithelium of atrophic type. The stroma was compact with densely placed cells. The muscular boundaries were clearly seen. The cervix and the vagina showed one row of columnar mucus-secreting cells, beneath which one or two layers of reserve cells were present.

Group VI. Castrated Animals Receiving Chorionic Hormone and Estrogen

There was no significant alteration in the gross appearance of the uteri in this group except moderate dilatation of the lumen. Although the estrogenic effect was apparent, it did not parallel the intensity seen in the castrated animals receiving only estradiol. The histological pattern was that seen during estrus, with areas of glandular cystic hyperplasia. Perinuclear vacuolation and squamous metaplasia were present in focal areas. The stroma was compressed, fibrotic, and rather acellular. The myometrium was unremarkable. The cervix and the vagina showed marked epithelial hyperplasia with keratinization.

Group VII. Castrated Animals Receiving Progesterone and Estrogen

The gross and microscopic findings in the uteri of these animals were similar to those described in Group I. Figure 9 illustrates the appearance of a surface epithelium and can be compared with Figures 4 and 5.

A minor difference was that the glands did not participate in the changes to the same extent as those in Group I. The alterations in the cervix and the vagina were also similar to those in Group I.

COMMENT

It is worth while mentioning that small variations in the degree of histological changes were observed within animals of the same group. This is consistent with the findings of other workers and emphasizes the importance of specific susceptibility of endocrine target organs.

The most interesting feature of this study has been the abnormal endometrial pattern

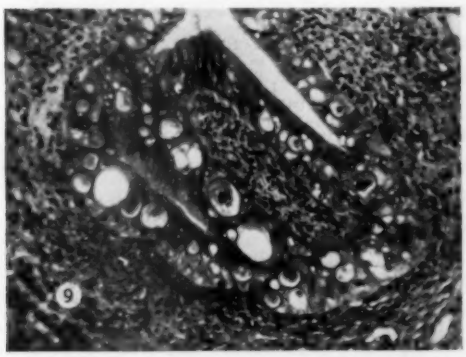


Fig. 9 (Group VII).—Castrated animal receiving progesterone and estrogen. Surface epithelium showing change similar to that illustrated in Group I. Hematoxylin and eosin; reduced ¼ from mag. × 140.

obtained by injecting normal animals with chorionic hormone and estradiol (Group I). A striking stimulating effect was noted in which marked secretory and proliferative activities occurred simultaneously. The secretory component took the form of an extensive process of marked vacuolation which led to the formation of the so-called intraepithelial cysts. In the group of normal animals receiving chorionic hormones alone, a normal progestational type of endometrium was obtained such as one would expect from the stimulating effect of the chorionic hormones on the ovaries. Here we observed that, in general, there was no epithelial vacuolation and that the secretory material, in contrast to Group I, was usually seen in the lumen of the glands, as if it were normally secreted. This led us to think that the gigantic vacuoles in the animals of Group I resulted from a process of overproduction and faulty secretion. Hence, a more appropriate designation would be "intraepithelial retention cysts." The reason for categorizing this feature of the change is that in the normal or castrated animals receiving estrogen alone these large retention cysts were practically absent and, therefore, in the present experiments their full development seemed to be dependent on more than one hormone.

Using a total dose of 50 mg. of estradiol injected in 10 days, followed by 100 I. U. daily of chorionic hormone for 5 days, Bourg, Simon, and Jaworski [14] obtained in normal rats vacuolar changes of the uterine epithelium which seem to us to represent the early phase, or a less developed stage, of the change we have now described. The authors thought that this alteration was a metaplastic degenerative cystic process and considered it to be of the same nature as that which they saw in adult rats as a result of juxtacervical ligature of the uterine horns and injection of estrogen, with or without progesterone. The vacuolation that we have seen occurring as a result of the administration of estrogen alone is mainly of the perinuclear type, confined to the cell, and forming what can be designated as intracytoplasmic vacuoles. As a rule, when the vacuoles become confluent and form large cysts, there are marked associated degenerative changes and heavy leucocytic infiltration. The severe estrogenic vacuolation, contrary to the epithelial retention cysts which occur throughout the endometrium, is seen mainly at the juxtacervical segment and involves rapidly into squamous metaplasia.

The fact that we have been able to reproduce a change very similar to that in Group I by injecting a large dose of estradiol and progesterone in castrated animals (Group VII) and the failure of the chorionic hormone alone (Group V) or in combination with estrogen (Group VI) to induce the

change in ovariectomized animals indicate that the role played by the chorionic hormone is exerted through the stimulation of the ovaries and the secondary release of high levels of progesterone. In preliminary studies, we have injected castrated rats with as much as twice the dose of estradiol used in the present experiments, during similar periods of time, and have not obtained the abnormal pattern now under discussion. That the change is not produced by the progesterone alone is apparent from our findings in Group II and, furthermore, from the experience of previous workers. Selye and coworkers, injecting as much as 4 mg. of progesterone daily to rats for 12 days, reported an endometrium of diestrous type, not frankly progestational. Thus, it seems that the intensive vacuolation and proliferative activity is the result of the concurrent action of the estrogen and progesterone. This would be a case similar to the synergistic effect of the progesterone and estrogen in the production of mucification in the vagina. Estrogen or progesterone alone is not able fully to induce this change, but they are, when administered together. In the same way the so-called intraepithelial retention cysts occur very rarely, or not at all, with estrogen or progesterone alone, but they are striking when these hormones act together at a given dose. It is surprising, however, that in the extensive literature concerning the cooperative and antagonistic effects of the progesterone and estrogen, which have been recently surveyed by Courrier,[16] no mention is made of the occurrence of this pathologic change. Selye, Browne, and Collip administered to castrated rats 30γ of estrone and 400γ of progesterone daily (estrogen : progesterone ratio, E/P—1/13) for 20 days and reported a "uterus in the second stage of progestational proliferation." Korenchevsky and Hall, who injected castrated rats simultaneously with different amounts of estrogen and progesterone, which varied from 1γ to 30γ of estrone and 400γ to 3000γ of progesterone, have not reported observations comparable to those in the present work. With the combination of 30γ of estradiol given three times per week and 3000γ of progesterone daily for 21 days, they observed a weak development of lace-like mucosa. It is our impression that their Figure No. 44, illustrating the appearance of the uterus with this latter treatment, shows minimal vacuolar change of the type we have described. We must keep in mind that with regard to the synergistic and antagonistic effects of estrogen and progesterone the importance of the relative and absolute levels of the hormones has been conclusively established.[16] Jones and Astwood,[19] injecting castrated rats simultaneously with varying quantities of estrogen and progesterone, demonstrated that, although at certain doses the progesterone was not sufficient to suppress the initial estrous smear, when the dosage of progesterone was adequate, the estrous smear was immediately transformed into the diestrous type and remained so throughout the injection period, regardless of the amount of estrogen given. They found that 1 mg. of progesterone counteracted as much as 100γ of estradiol (E/P—1/10). Alloiteau [20] has shown that with 1250γ of estradiol and 1250γ of progesterone (E/P—1/1) the rats showed vaginal mucification. Another example of the importance of the relative levels of the hormones is given by the effect of estrogen in the placentomata formation induced by progesterone.[21] Our findings indicate that at the doses of 52.5γ of estrogen and 2000γ of progesterone (E/P—1/38), (Group VII), the progesterone enhanced and modified the vacuolating action of estrogen on the endometrial mucosa and led to the formation of the intraepithelial retention cysts.

Although we have not seen any trace of squamous transformation in the uteri of any of our animals of Group I, it remains to be seen whether continuous stimulation over longer periods of time would ultimately lead to this form of metaplasia. Previous observations have shown, however, that progesterone antagonizes the squamous metaplasia induced by estrogen.[22]

The proliferative activity in the animals of Group I was manifested by the presence of frequent mitosis, formation of cellular aggregates, and moderate glandular hyperplasia. We should note, however, that in Group I, as well as in Group VII, there was not, in general, cystic glandular hyperplasia, so the antagonistic effect of the progesterone to this particular estrogenic action was apparent.

Another interesting feature in Group I was the morphology of the nuclei in the proliferated epithelial cells. Although frequently distorted by the secretory vacuoles, the nuclei showed a characteristic vesicular and swollen appearance and occasionally, in focal groups of cells, displayed an unusual large size.

The findings described in the normal animals receiving chorionic hormone and estrogen give further support to the idea previously expressed concerning the pathogenesis of the abnormal endometrial pattern found in certain cases of endometrial abortion, hydatidiform mole, chorioepithelioma, etc. In other words, the nuclear changes and the simultaneous occurrence of abnormal proliferative and secretory activities in some of these cases could be traced to hormonal actions.

The results in Group V are pertinent to the discussion as to whether or not the chorionic hormones stimulate the release of estrogenic substances from the adrenal glands.§ At the dosage used there was no histological evidence of any stimulating effect exerted by the chorionic hormones directly on the uterine epithelium or of any extragenital secondary stimulation.

The observation on the different responsiveness of the exocervical and endocervical segments to the various hormonal stimuli used merits further study.

SUMMARY

The administration of human chorionic hormone and estrogen to normal rats produces an abnormal endometrial pattern characterized by the simultaneous occurrence of proliferative and secretory changes. The nuclei of the epithelial cells show, in general, a more vesicular appearance than normal and occasionally display a larger size. The abnormal pattern can not be induced by treating normal rats with estrogen or chorionic hormone alone, or by administering both hormones into castrated animals. A somewhat similar alteration is seen, however, when oophorectomized rats are treated with large doses of estrogen and progesterone.

The pathogenesis and the possible relation of this alteration to certain endometrial changes seen in humans are briefly discussed.

This investigation was supported in part by research grant C-1345 from the National Cancer Institute of the National Institutes of Health, U. S. Public Health Service, and by a grant from the Damon Runyon Memorial Fund, and was begun when the author was a Kellogg Foundation Fellow in Pathology, Memorial Center for Cancer and Allied Diseases.

Opportunities and encouragement in the pursuance of this work were given by Dr. Fred W. Stewart and Dr. Robert C. Mellors. Dr. Oscar Miro-Quesada Jr. assisted in the preparation of this paper.

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Endometrial Changes in the Rat

The Effect of Estrogen When Administered After an Interval Following Castration

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Differences exist in the endometrial response to estrogens in castrated rats treated immediately following oophorectomy and in those in which the hormone is administered after an interval period. Migliavacca,[1] using six rat units of estradiol administered daily for 21 days to rats which had been previously castrated for 4 weeks, reported the occurrence of focal areas of syncytial proliferation in the uterine epithelium. He referred to this change as "syncytial transformation of the uterine epithelium" and viewed it as resulting from the simultaneous actions of the estrogen plus the gonadotropic hormone produced in the pituitary in response to castration.

The present experiment compares endometrial changes in oophorectomized rats receiving the same dosage of estrogens at different intervals of the postoperative period.

MATERIAL AND METHOD

Fourteen spayed white female rats, 3 to 5 months old, of the Sherman strain formed the experimental group. Estradiol dipropionate (Schering) was administered for 20 days, beginning 4 weeks after oophorectomy, at the dose of 35γ daily. The procedure followed was identical to that described previously.[2] The results in the experimental animals were compared with the findings in similarly treated normal rats and castrated rats in which injections were started one day after ovariectomy.[2] These last two groups of animals will be referred to as the controls.

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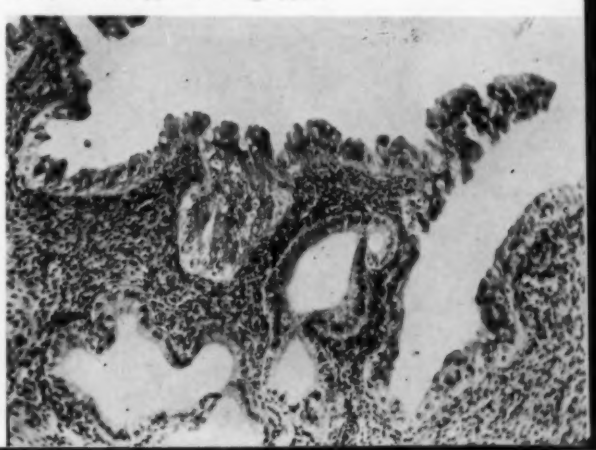
RESULTS

There was no significant difference in the gross appearance of the uterus in the experimental and control animals. In both, a moderate dilatation of the lumen was observed. Frequently, in the experimental group, the luminal dilatation was more marked in one horn. Histologically, there were several interesting differences. First, it was noted that the glandular hyperplasia was less marked in the experimental animals. The cystic tendency of the glands, which was so striking in the ovariectomized animals treated immediately after castration, was lacking in the experimental animals. In our experience, the occurrence of focal areas of proliferation in the uterine epithelium, displaying the appearance of syncytial masses (due to the reduplication of cells and partial blurring of the protoplasmic limits), was not an exclusive feature of the experimental animals, but was also occasionally seen in the controls. However, in the experimental group this feature was more frequent and extensive. In some of the experimental animals the formation of epithelial aggregates gave

Fig. 1.—Glandular hyperplasia. Focal areas of syncytial proliferation of the surface epithelium, growing down into the stroma or protruding toward the lumen in a papillary fashion. Hematoxylin and eosin; reduced ¼ from mag. × 75.



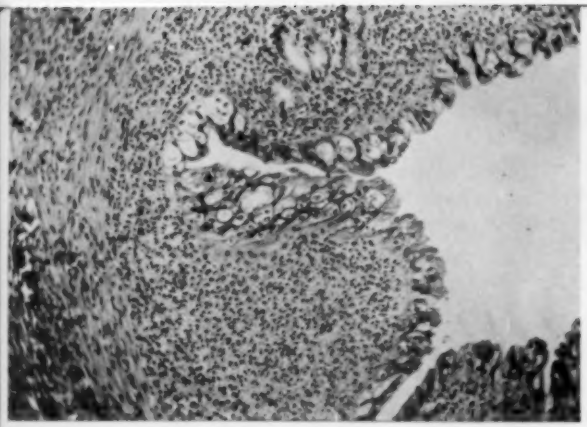


Fig. 2.—Marked vacuolation and aggregation of cells in focal areas are illustrated. The basal membrane is lost at the level of epithelial proliferation. Hematoxylin and eosin; reduced ¼ from mag. × 75.

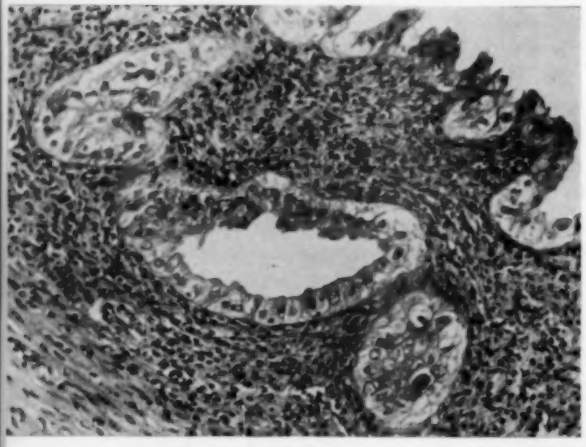
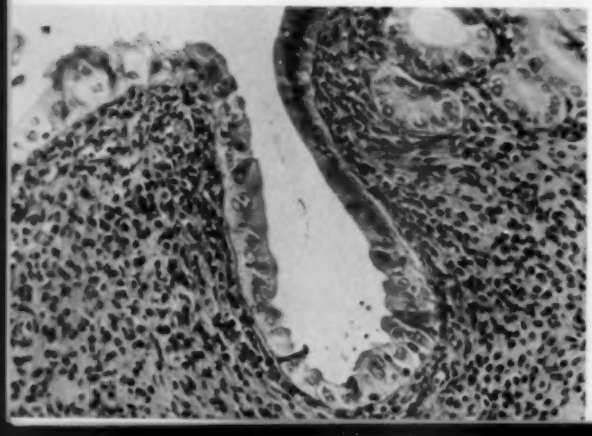


Fig. 3.—Marked cytoplasmic vacuolation involving surface and glandular epithelium. Scattered large nuclei can be seen. Hematoxylin and eosin; reduced ¼ from mag. × 180.

rise to compact papillary masses of cells which, without a core of accompanying stroma, protruded into the lumen or grew down into the stroma (Figs. 1 and 6) and,

Fig. 4.—Uterine surface showing transition from normal (right) to altered epithelium. Large cells with hypertrophic nuclei are conspicuous. Hematoxylin and eosin; reduced ¼ from mag. ×180.



in some cases, formed sheets of cells which could be seen floating free in the lumen. As demonstrated in Figures 2 and 3, the degree of epithelial vacuolation frequently surpassed that seen in the controls and formation of large epithelial cysts was observed in scattered areas. In these vacuoles occasional centrally located hyaline masses were noted which stained with the periodic acid technique and mucicarmine, even after diastase treatment. We have not seen the occurrence of pseudoglands as a result of the vacuolation, as reported by Migliavacca. Finally, throughout the mucosa, focal areas were seen in which the epithelial cells showed deep eosinophilic protoplasm and hyperchromatic nuclei which, on the average, were of a larger size than in the controls. This latter feature was rather striking when there was also marked squamous metaplasia. A clear contrast could be noted in some instances between the altered and adjacent normal epithelium (Figs. 4 and 5). In some cases there was only nuclear enlargement without cytoplasmic hypertrophy. In the case illustrated in Figures 6 and 7, unusually large nuclei were seen in focal areas adjacent or not to zones of squamous metaplasia. In 7 of the 14 experimental animals foci of squamous metaplasia were seen in the endometrium. Beneath the surface epithelium the stroma tended to show marked hyalinization, and in focal areas this process seemed to involve the more deeply situated epithelial cells. In general, the uterine stroma was densely infiltrated by leucocytes. The myometrium was unremarkable. The cervix and the vagina showed marked keratinization but only moderate epithelial proliferation.

COMMENT

We have no definite explanation accounting for the histological differences in the experimental and control animals. It is likely that the less developed glandular hyperplasia and the lack of cystic glandular dilatation seen in the animals treated after a period of castration could simply be due to the fact that after four weeks of ovariectomy

the uterus has undergone marked involution and atrophy. Therefore, the initial effect of estrogen is to reverse the state of atrophy to one of normalcy. Migliavacca advanced the idea that the syncytial transformation of the uterine epithelium is the result of the estrogen given plus the action of the gonadotrophins formed in response to oophorectomy. We have examined female rats of the same age and strain as that of our experimental group four weeks after castration and found that by this time the pituitary shows a noticeable hyperplasia of basophiles of the type currently designated as "gonadotrophs." [3] In the experimental animals, these cells have considerably diminished as a result of the estrogen given. Fluhmann has recently questioned the necessity of the pathogenetic mechanism proposed by Migliavacca, pointing out that the syncytial-like transformation can result from the action of estrogen alone. We have already indicated that this change was also noted in our control animals, but that it was far more intensive and frequent in the experimental group. Further investigations are necessary for complete elucidation of this point. Little can be said in connection with the unusual nuclear enlargement seen in some of the experimental animals. As previously stated, the impression was gathered that this change was related to the process of squamous metaplasia induced by the estrogen. Whether or not this nuclear change is specific for the experimental setting now used can not be decided at present. In any case, it is interesting to point out that, although squamous metaplasia was also seen in the control animals, in no case was there to be found unusual nuclear enlargement of the type seen in the experimental group. Furthermore, previous studies on squamous metaplasia of the uterus induced by hormones in rats,* as well as the studies on the histogenesis of squamous metaplasia, do not mention this type of nuclear alteration.† With reference to this problem it should be remembered that it

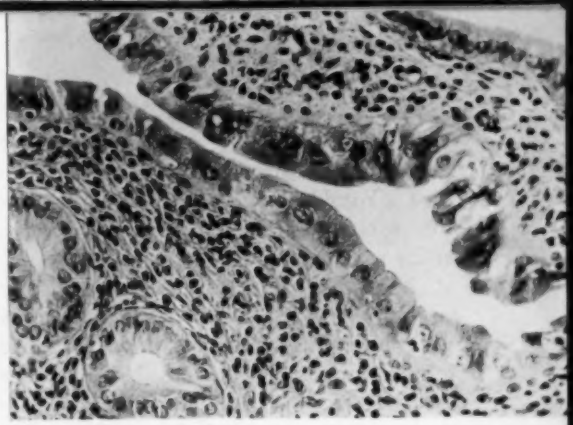


Fig. 5.—Another view illustrating the contrast between normal (upper right) and altered epithelium. Hematoxylin and eosin; reduced ¼ from mag. ×190.

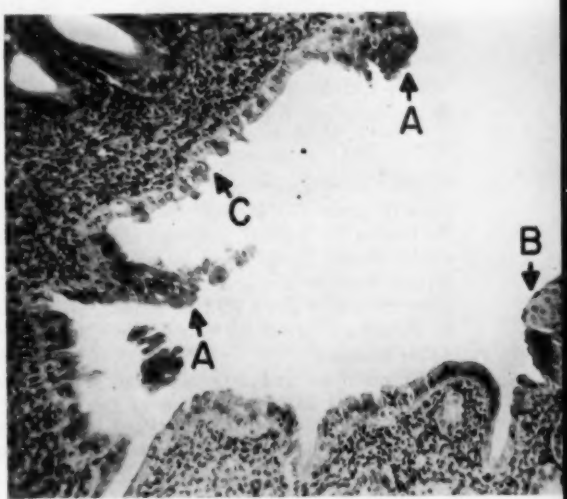
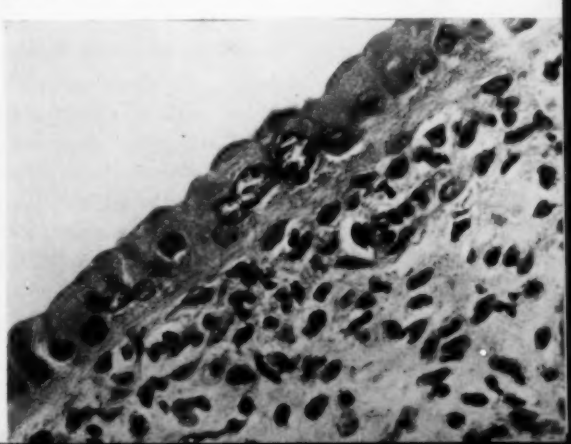


Fig. 6.—Panoramic view showing, *A*: syncytial masses of proliferated cells; *B*: focus of squamous metaplasia in area of nonaltered epithelium, and *C*: hypertrophic nuclei.

Fig. 7.—High-power view showing group of enlarged nuclei among others that are normal. Hematoxylin and eosin; reduced ¼ from mag. × 400.



* References 5 and 6.

† References 4, 7, 8, and 9.

has recently been demonstrated that estrogens elicit a considerable increase in the nuclear volume of some cells of the uterine epithelium in the mouse.[10]

The occasional occurrence of unusually large nuclei in the uterine epithelium under the experimental conditions described gives further support to the possible endocrine basis of the nuclear abnormalities recently shown to be present in human endometrium in association with conditions where chorionic tissue is also present.[11]

SUMMARY

The histological differences seen in the endometrium of rats treated with estrogen immediately after castration and in those in which the hormone is given after a four-week interval following oophorectomy are described.

This investigation was supported in part by research grant C-1345 from the National Cancer Institute of the National Institutes of Health, U. S. Public Health Service, and by a grant from the Damon Runyon Memorial Fund, and was begun when the author was a Kellogg Foundation Fellow in Pathology, Memorial Center for Cancer and Allied Diseases.

Opportunities and encouragement in the pursuance of this work were given by Dr. Fred W. Stewart and Dr. Robert C. Mellors. Dr. Oscar Miro-Quesada Jr. assisted in the preparation of this paper.

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Glomerulotubular Nephrosis Correlated with Hepatic Lesions

I. A Morphologic Investigation of the Changes of Progressive Autolysis in Human, Rabbit, and Rat Tissues

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and
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The similarity of premortem degenerative changes and postmortem autolytic changes in the renal parenchyma is well recognized.* It became essential to distinguish between these types of change in an investigation of the correlation of premortem hepatic and renal damage in human autopsy material. Many excellent studies comparing premortem and postmortem alterations have been made on both the morphological † and chemical aspects,[5] but it was not possible to apply the findings of these studies to the particular problem under consideration. The following studies were, therefore, directed to obtaining information necessary to the investigations noted above.

MATERIALS AND METHODS

In the present study of postmortem changes in the liver and kidney of man, it was not possible to take serial sections from the organs over any long period of time while leaving them in their natural environment. Initial control sections were needed from each case to compare with later postmortem changes. To accomplish this, control material was taken for fixation immediately on removing the kidney and liver from the body. Further blocks were removed from these organs at predetermined intervals, keeping the organs at room temperature, moist, and free of gross contamination. In this regard the early work of Cruickshank [3] demonstrating the striking effect of bacteria on the autolytic process influenced our choice of a nonsterile environment such as exists in the body after death. We were also influenced to use a method in the present study that would make our results applicable to the later study of minimal pathologic alterations of human material which contains a variable amount of postmortem change. For comparison with these changes in man, rabbit and rat tissues were studied under the same circumstances. The control sections of animal tissues were placed in fixative within five minutes after death.

All blocks for histological study were removed in an identical fashion. They were excised with a sharp knife, using a minimum of pressure, and immediately placed in an abundant amount of fixative. After fixation they were dehydrated, cleared, and embedded, with use of identical schedules. Staining was carried out so that all sections to be compared were stained under identical circumstances. A survey of many routine stains was made with regard to selectivity in the detection of various stages of antemortem change in the epithelium of the nephron from the earliest type of dysadaptation to frank necrosis. This led to the use of hematoxylin and eosin and Masson's trichrome stains on 5μ paraffin sections.

EXPERIMENT 1: *Autolytic Changes of Rabbit Kidneys Removed by Nephrectomy.*—Procedure: The left kidney was removed under ether anesthesia from each of 10 apparently healthy adult rabbits of both sexes and of average weight of 3 kg. The kidneys were hemisectioned by median longitudinal in-

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Medical Research Fellow, National Research Council of Canada (Dr. Crowson).

From the Departments of Pathology, Queen's University and the Kingston General Hospital, Kingston, Ont., Canada, and the University of Edinburgh, Scotland.

* References 1 and 2.

† References 3 and 4.

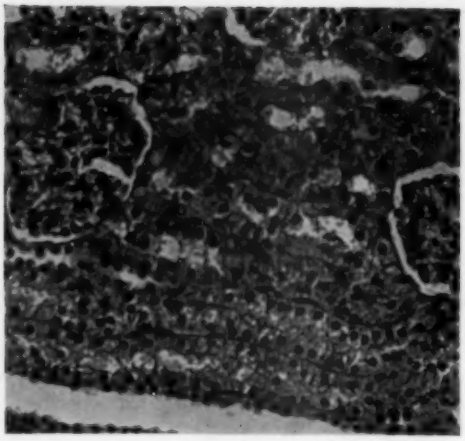


Fig. 1 (CR 5).—Rabbit kidney. Control, no autolysis. Hematoxylin and eosin; reduced slightly from mag. × 300.

cision and placed in bottles containing isotonic saline for storage at room temperature (15C).‡ Vertical sections were removed from each specimen, commencing from one pole, and placed in 10% formalin § at time intervals of 0, 3, 7, 24, and 74 hours, respectively.

Observations: In the hematoxylin and eosin stained sections, there was noted a progressive depletion of cytoplasmic and nuclear substances in the epithelium of the proximal convoluted tubules (Figs. 1 to 5, inclusive). After three hours, no change was seen. After seven hours, a definite tinctorial loss was seen in both cytoplasm and nuclei. The luminal borders became more ragged and the cells appeared swollen. There was minimal perinuclear vacuolation in the epithelium of the proximal tubules, while the glomeruli and the distal and collecting tubules appeared unaffected as yet.

At the end of 24 hours, many further changes had developed. Cytolysis and karyolysis had progressed to a state of partial disruption of the proximal epithelium with detachment of cells and intraluminal accumulation of esoinophilic, granular, and cellular debris. Many nuclei had disappeared or remained only in shadow form. A granular, eosinophilic material appeared in the glomeruli, partially obliterating the capsular space. In contrast to the surrounding tubal epithelium, the glomerular nuclei stood out in pyknotic relief. The interstitium was just commencing to show edematous distention, forming tiny clear spaces containing a pale pink material. Nuclear pyknosis had now commenced in the epithe-

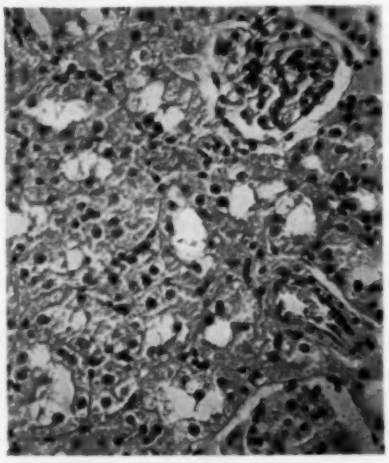
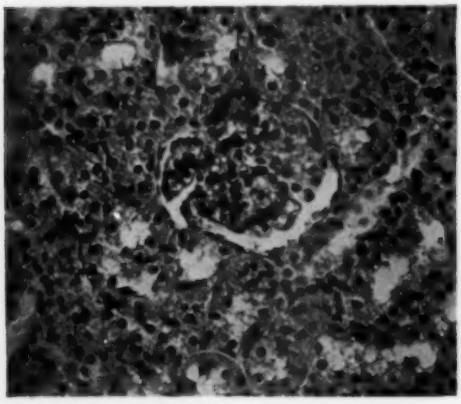


Fig. 3 (CR 5).—Rabbit kidney; 7 hours' autolysis. Nuclear and cytoplasmic pallor in proximal tubules with perinuclear halos. Hematoxylin and eosin; reduced slightly from mag. × 300.

‡ Room temperature of 15 C was chosen in preference to body temperature in order to delay bacterial growth in the nonsterile tissues.

§ 10% formalin was employed in this portion of the study to conform with techniques of the human autopsy material.

Fig. 2 (CR 5).—Rabbit kidney; 3 hours' autolysis. No perceptible changes are noted. Hematoxylin and eosin; reduced slightly from mag. × 300.



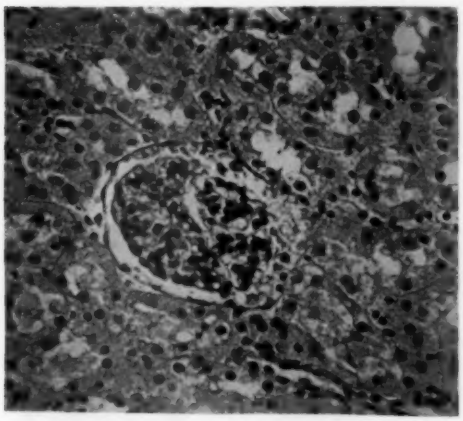
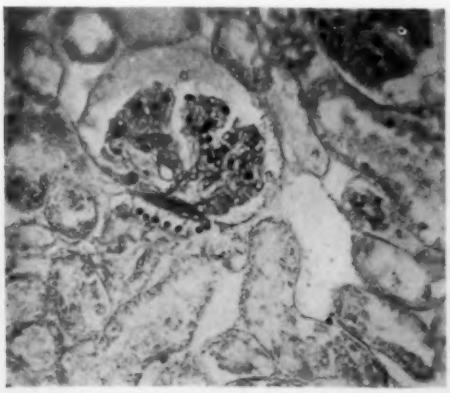


Fig. 4 (CR 5).—Rabbit kidney; 24 hours' autolysis. Fluid and debris in various lumina. Some further tinctorial loss and commencing disruption in proximal tubules. Pyknosis of glomerular, interstitial, and distal tubular nuclei. Hematoxylin and eosin; reduced slightly from mag. × 300.

lium of the distal portions of the nephron, and the staining properties of the cytoplasm had changed from a faint to a pronounced acidophilia. The cells appeared swollen, and the lumina contained eosinophilic granular debris. After 74 hours of autolysis, little recognizable renal structure remained. The changes may best be summed up as persistence of the basement membranes of the entire nephron; loss of all nuclear and cellular structure in the proximal tubules and loops

Fig. 5 (CR 5).—Rabbit kidney; 74 hours' autolysis. Basement membranes persist throughout. Some pyknotic nuclei remain in the glomeruli and distal tubules. Nuclear and cytoplasmic detail has disappeared in the proximal tubules. Gross interstitial edema is present. Hematoxylin and eosin; reduced slightly from mag. × 300.



of Henle; gross edema of the interstitium, and the rare persistence of some distorted nuclear and cellular detail in the glomeruli and distal nephron. Pyknosis, while rarely seen in the upper portion of the nephron, was a feature of the remaining nuclei of the glomeruli and of the distal and collecting tubules. In addition, the cytoplasm of the distal tubules tended to become more densely

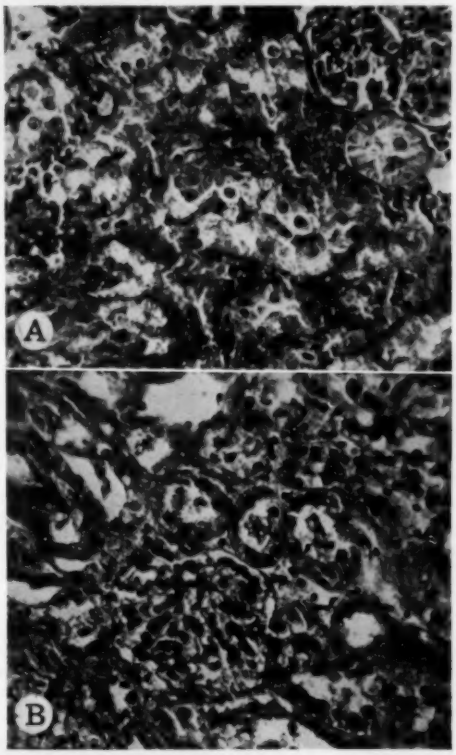


Fig. 6 (CR 1).—Rabbit kidney. *A*, Control section; no autolysis. Many degenerating cells with pyknosis and intense acidophilia are noted in the proximal tubules. Trichrome stain; reduced slightly from mag. × 300. *B*, Twenty-four hours' autolysis. The nuclear and cytoplasmic changes persist in easily recognized fashion.

eosinophilic. Similar changes were noted with the trichrome stain.

In 2 of the 10 rabbit kidneys, nuclear pyknosis and cytoplasmic degeneration in the epithelium of the proximal convoluted tubules and epithelial casts were observed in the control sections taken immediately after nephrectomy. As autolysis proceeded,

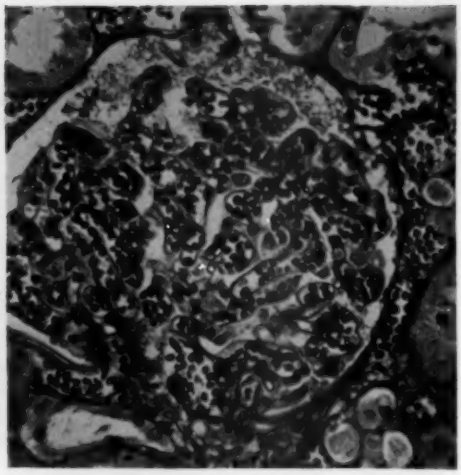


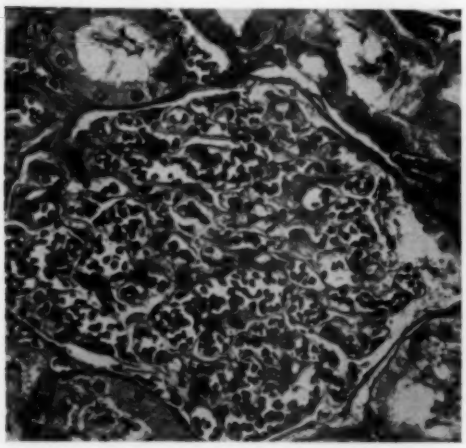
Fig. 7 (A-487).—Human kidney. Control section taken one and one half hours after death, with pyknotic nuclei and hyperchromatic cytoplasma, plus casts. Trichrome stain; reduced slightly from mag. × 300.

these premortem degenerative changes stood out in sharp contrast to autolyzing normal philia of the cytoplasm persisting until the stage of general dissolution, at which point differentiation became impossible (Fig. 6*A* and *B*).

EXPERIMENT 2: *Autolytic Changes in Human Kidney and Liver Tissue.*—Procedure: A 500 gm. block of liver and one entire kidney were removed at autopsy. One block from each liver and kidney was placed in 10% formalin, and the remaining tissue wrapped in toweling soaked in isotonic saline and allowed to lie at room temperature (15 C), with random samplings over variable intervals of time in 10% formalin. A total of six autopsy cases were studied in this fashion.

Observations: The renal changes observed over a wider range of time intervals

Fig. 8 (A-487).—Human kidney; 27½ hours' autolysis. The premortem nuclear and cytoplasmic changes are seen to persist, in the company of cytokaryolysis and fluid imbibition. Trichrome stain; reduced slightly from mag. × 300.



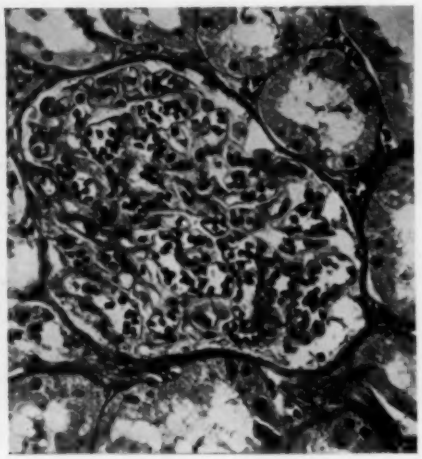


Fig. 9 (A-487).—Human kidney; 57½ hours' autolysis. The degenerative premortem changes can still be identified in the proximal tubules. Active nuclear and cytoplasmic lysis is seen elsewhere. A small fragment of distal tubule appears at the left with densely pyknotic nuclei and dark-staining cytoplasm. Trichrome stain; reduced slightly from mag. × 300.

Fig. 10 (A-487).—Human liver. Control section, 1½ hours post mortem, showing sinusoidal congestion. Trichrome stain; reduced slightly from mag. × 300.

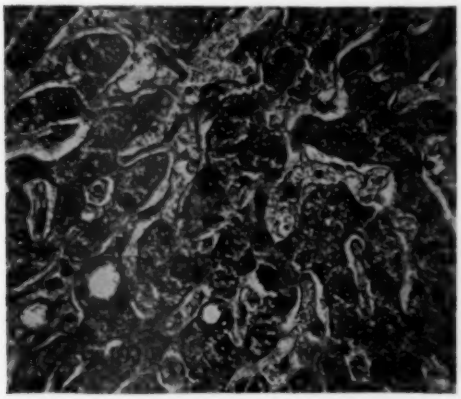
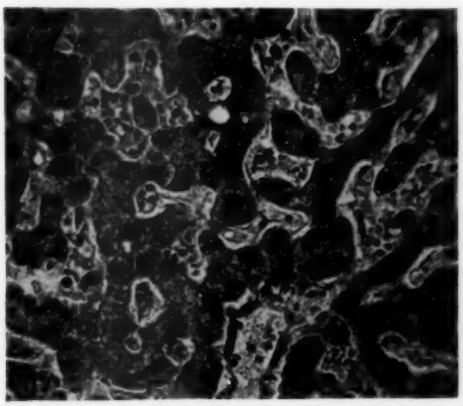


Fig. 11 (A-487).—Human liver; 27½ hours' autolysis. An increase of fluid and amorphous debris in the sinusoids and a slight coarsening of the granularity of the cytoplasm are the only noteworthy features at this period. Trichrome stain; reduced slightly from mag. × 300.

correspond closely with those seen in the rabbit kidneys. There was less tendency toward complete dissolution and toward imbibition of fluids into capsular spaces and tubal lumina, presumably owing to the drier conditions of storage. Again, as in the case of the rabbits, if premortem changes were present in control sections, they could be traced throughout the periods of autolysis, necrotic epithelial cells retaining the dense chromatophilia of cytoplasm and nucleus. The number of pyknotic nuclei in the proximal tubules appeared to remain constant, while the total number of nuclei decreased. Such a case, A-487, is shown in Figures 7, 8, and 9.

Fig. 12 (A-487).—Human liver; 57½ hours' autolysis. There is slight shrinkage of the cell volume and an increase in the number of pyknotic nuclei. The cytoplasm of the parenchymal cells is stained more intensely. Trichrome stain; reduced slightly from mag. × 300.



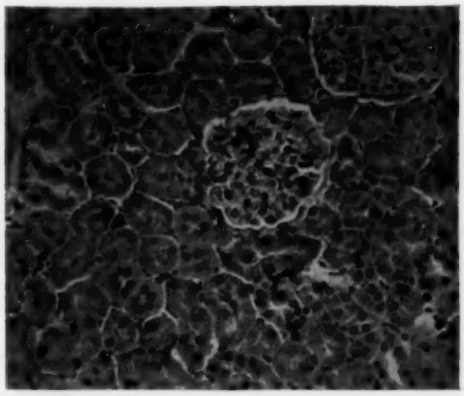
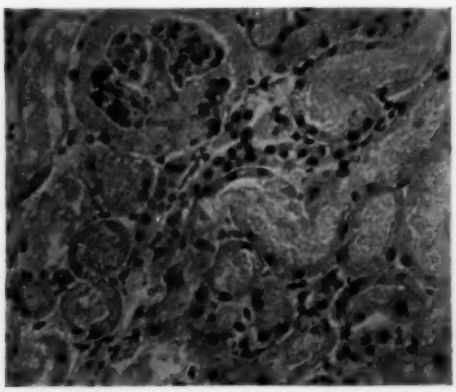


Fig. 13 (C 4).—Rat kidney. Control; no autolysis. Hematoxylin and eosin; reduced slightly from mag. × 300.

The hepatic changes were more variable. In four of the six livers there was noted chromatin condensation in the parenchymal nuclei, with an over-all increase in the number of pyknotic and karyorrhectic nuclei. The remaining two livers in this group revealed, instead, progressive karyolysis. Cytoplasmic changes were constant in all livers, consisting

Fig. 14 (C 4).—Rat kidney; 20 hours' autolysis. All lumina are distended with a debris-laden fluid. There is much evidence of cytokaryolysis. The number of pyknotic nuclei in the identifiable proximal tubules appears identical with that of the control section. Pyknosis is mainly confined to glomeruli and distal tubules. Marked interstitial distention is seen. Hematoxylin and eosin; reduced slightly from mag. × 300.



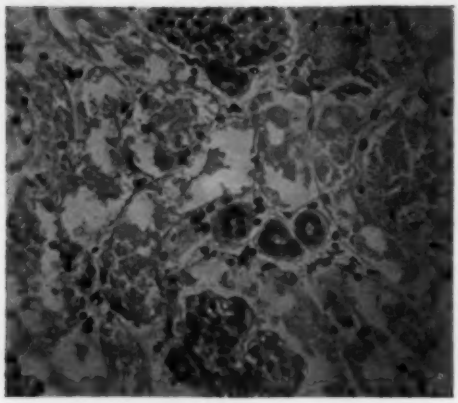


Fig. 15 (C 4).—Rat kidney; 44 hours' autolysis. Cytokaryolysis has been completed in the proximal tubules, leaving only granular debris. Distal tubular, interstitial, and glomerular nuclei remain pyknotic, and the cells are partially disrupted and separated from their basement membranes. Hematoxylin and eosin; reduced slightly from mag. × 300.

of shrinkage of total cell volume and increased staining intensity in the later phases of autolysis. There was minimal imbibition of fluid into the sinusoids and the branching pattern of the hepatic cords remained intact as late as 70 hours post mortem (Figs. 10, 11, and 12, liver from the same case as kidney in Figs. 7, 8, and 9).

EXPERIMENT 3: *Autolytic Changes in Rat Kidney and Liver Tissues.*—Procedure A: Ten healthy Wistar strain albino rats of both sexes and of average weight of 175 gm. were killed by excising the kidneys and liver under ether anesthesia. Blocks of kidney and liver were placed in isotonic saline and allowed to autolyze at room temperature (15 C). Samples were fixed in Helly's fluid at 0, 20, 44, and 96-hour intervals. In addition to the routine hematoxylin and eosin and trichrome stains, sections from two rats were followed by the periodic acid-Schiff stain (according to McManus [6]).

Observations: The renal changes paralleled those of the rabbit kidneys, with progressive lysis of cytoplasm and nuclei of the proximal convoluted tubules, while the distal convoluted tubules showed an initial nuclear pyknosis and cytoplasmic acidophilia and

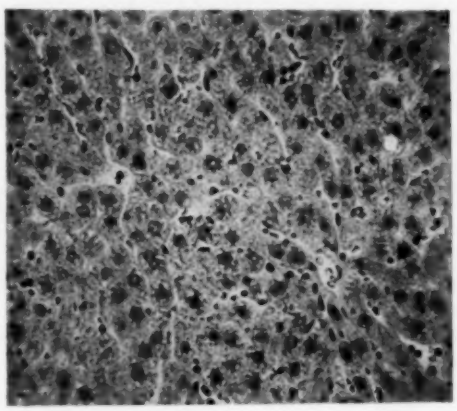
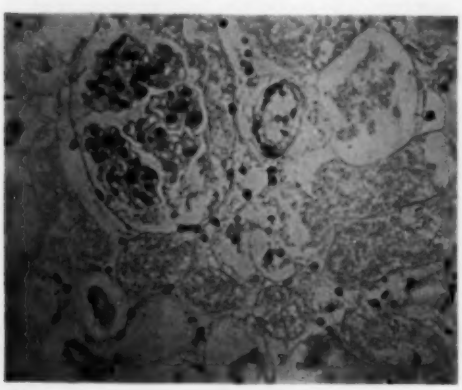


Fig. 17 (C 4).—Rat liver. Control; no autolysis. Hematoxylin and eosin; reduced slightly from mag. × 300.

Fig. 16 (C 4).—Rat kidney; 96 hours' autolysis. Basement membranes, pyknotic glomerular, interstitial, and rare distal tubular nuclei are all that remain of recognizable renal tissue. Hematoxylin and eosin; reduced slightly from mag. × 300.



density. In addition, a brownish, granular material tended to concentrate at the basal edge of the proximal tubular epithelium in the 20-hour specimen, later to disappear completely (Figs. 13 through 16).

The hepatic changes consisted of progressive shrinkage of the cord cells evenly throughout the lobule, plus fluid imbibition into sinusoids and the gradual accumulation therein of an amorphous eosinophilic material. The cytoplasm of the parenchymal cell during the first 20 hours of autolysis revealed increased pallor of staining, and the nuclei were undergoing karyolysis. A rather striking reversal of nuclear and cytoplasmic staining was noted beyond this time interval,

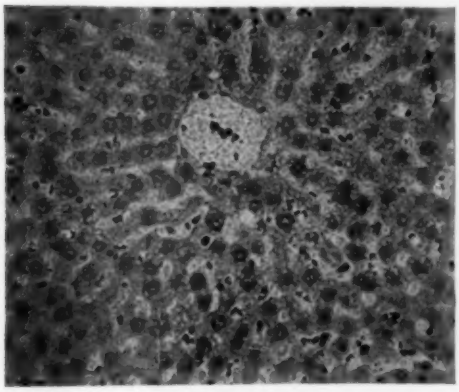
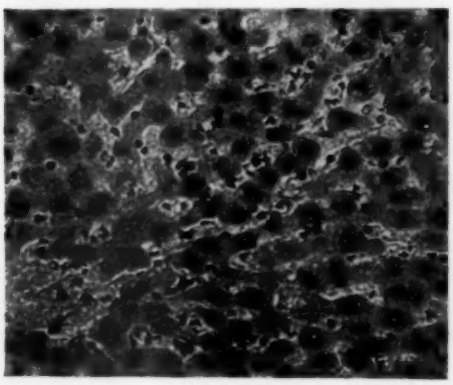


Fig. 18 (C 4).—Rat liver; 20 hours' autolysis. There is a slight shrinkage of the cord cells plus generalized cytokaryolysis and imbibition of fluid into the sinusoids. Hematoxylin and eosin; reduced slightly from mag. × 300.

consisting of a decided increase in density of the cytoplasmic contents displaying a peculiar "ground glass" granularity, associated with a minor decrease in the cell volumes and darkening of nuclear chromatin structure in the intact nuclei. The latter approached pyknosis and/or karyorrhexis in several instances (Figs. 17 through 20). There was no apparent accumulation of stainable fluid in the spaces of Disse. In the liver cells, periodic acid-Schiff positive material was found in the 0 and 20-hour specimens, but was negative beyond 20 hours.

Fig. 19 (C 4).—Rat liver; 44 hours' autolysis. Increased density of the nuclei and cytoplasm appears in striking contrast to Figure 18. The change is likened to "ground glass." Hematoxylin and eosin; reduced slightly from mag. × 300.



Procedure B: Four 300 gm. male Wistar rats were killed by an overdose of ether, and the right kidneys and a small portion of the livers were excised as controls through small right lateral incisions with a minimum of manipulation. The holes were plugged with absorbent cotton and the carcasses allowed to lie at room temperature (15 C), one being chosen on each successive day, from which the left kidney and a further portion of liver tissue were excised. Blocks of all tissue were fixed immediately in Helly's fluid. The investigations thus covered periods of 0, 24, 48, 72, and 96 hours.

Observations: All rats revealed a slight degree of intrinsic renal disease, as evidenced

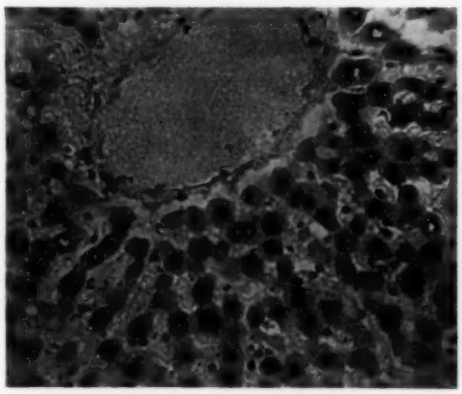


Fig. 20 (C 4).—Rat liver; 96 hours' autolysis. Pyknosis and karyorrhexis are seen in a few nuclei and the density of the cytoplasm remains unchanged. Despite marked sinusoidal distention the lobular structure remains intact, with no disruption of the cords. Hematoxylin and eosin; reduced slightly from mag. × 300.

by an unusually large number of degenerated cells in the distal and proximal convoluted tubules of control sections of the kidneys. The lesions were most marked in the rat employed for the 72-hour specimen, where protein exudate was found in the capsular spaces and tubal lumina in addition to acute degenerative changes in the epithelial cells.

The picture of autolysis in the kidney in hematoxylin and eosin sections followed closely on that seen in saline autolysis of Procedure A with a few important alterations, probably dependent upon moisture con-

tent and bacterial contamination. All former features were present, but on a reduced scale. The progress of autolysis was much delayed, fluid imbibition into all types of lumina was minimal, and the initial brownish, granular changes in the basal portion of the epithelia were more intense, lasting for 48 hours. After this period, however, cytokaryolysis progressed actively in the cortical regions, with loss of all cellular structure in the proximal tubules and disruption and nuclear pyknosis in the distal tubules and glomerular tufts. In small patches of superficial cortex and in the corticomedullary zone, there was partial persistence of nuclear and cellular detail in all components of the nephron as late as 96 hours. In sections stained by Masson's trichrome, premortem cytoplasmic degenerative changes could be recognized as late as 48 hours post mortem, after which the bright red smudgy or granular pigment became progressively browner and nonspecific. The pyknotic nuclei, however, persisted into the stage of general dissolution. At no stage did the postmortem autolytic process in the proximal tubules mimic premortem changes.

In the liver the changes were identical with those of saline autolysis apart from the relatively slight amount of fluid which was imbibed into the sinusoids in Procedure B.

COMMENT

The correlation of structural alterations in disease with the newer knowledge of physiological alterations requires the differentiation between degenerative premortem morphological change of all degrees and postmortem autolysis. It was not feasible to make a study of prolonged postmortem autolysis under the exact conditions which obtain in organs in the body after death. The present experiments are only valid as a method of studying postmortem change if we assume the quality of postmortem alteration is identical, whether autolysis is occurring in organs in the body or in organs removed from the body and maintained at room temperature, moist and free of gross contamination. Comparison of postmortem changes in Experiments 3A and 3B indicates this assumption to be correct. Rate of change after death was not considered important as a measure of whether observed alterations represent premortem or postmortem changes, because all conditions which determine the speed of postmortem autolysis are not known. It, therefore, seemed necessary to find some qualitative structural difference as a basis for differentiating premortem and postmortem changes.

In considering the results of this work, it must be borne in mind that postmortem changes not only occur in all normal tissues, but that postmortem changes are superimposed on any premortem changes which may be present.

In the above experiments it was possible to denote in the kidneys qualitative differences between purely postmortem alterations and alterations that consisted of premortem changes, plus whatever postmortem autolysis occurred after the control sections were taken. These differences were reported in the following manner: Pure postmortem alterations of proximal convoluted renal tubules followed the pattern of cytokaryolysis, a progressive depletion of cytoplasmic and nuclear contents. This change was modified in the case of the rat by a preliminary basal concentration of brownish pigment in the cytoplasm, which subsequently dissolved away. (A similar pigment is often seen in autolytic human kidneys but failed to manifest itself in the cases chosen for this study.) However, in tissues where the control sections showed premortem changes of nuclear pyknosis plus an increased density and eosinophilia of the cytoplasm, these changes persisted and were accentuated by postmortem autolysis up to the later stages of lysis. In the distal convoluted tubules, on the other hand, the normal course of postmortem autolysis was nuclear pyknosis and increased density and eosinophilia of the cytoplasm. But in some tissues the control sections obtained at the time of death showed patchy similar changes interpreted as premortem. These changes remained in advance of the similar

qualitative postmortem changes of the distal convoluted tubules for some time.

From these observations it seems possible to conclude that pyknosis and density, with acidophilia of the proximal convoluted tubules, always represent a premortem change. On the other hand, in the distal convoluted tubules, nuclear pyknosis and acidophilic density of the cytoplasm is the normal first stage of postmortem autolysis, and premortem changes can be diagnosed only by the presence of patchy areas showing this change well in advance of the general alterations.

This study was concerned with the analysis of minute cytological differences in order to provide an accurate means of correlating minimal premortem changes with metabolic disturbances. In seeking methods which might accentuate or bring out the differentiation between premortem or postmortem alterations, various stains were used. Many of the changes described were missed or poorly defined in hematoxylin-eosin stained sections. However, Masson's trichrome stain proved to be selective and indispensable in recognizing minute variations in cytological structure and indicates that this is an excellent method of differentiating on tinctorial grounds small variations in cytologic structure that might be missed by the use of the usual routine stains.

In the liver, the differentiation between premortem and postmortem alterations was less successful than in the case of the kidneys. The autolytic changes noted in the majority of the human and all the rat livers followed the sequence of initial cytokaryolysis and subsequent increase in density of nucleus and cytoplasm. At first, the nucleus and cytoplasm of the parenchymal cell grew paler and more indistinct, and there was a perceptible shrinkage of the cell volume. Later, the cytoplasm increased in density and chromatin condensation occurred in the nuclei, resulting in a hardened "ground glass" appearance. This alteration was found to coincide with a disappearance of glycogen, as estimated by the periodic acid-Schiff reaction, so that we interpret the shrinkage in cell volume to be partially a result of glycolysis. In this regard Morrione and Mamelok [7] have demonstrated the persistence of minute but stainable quantities of glycogen in human liver up to 48 hours post mortem. Throughout the entire period of autolysis there was a progressive accumulation of amorphous debris and fluid in the sinusoids, but even after 96 hours' immersion in saline no disruption of the branching pattern of the lobular cords occurred. On the other hand, van Beek and Haex [8] have demonstrated a progressive postmortem lobular disruption in a case of subacute hepatic necrosis, suggesting that autolysis may disrupt liver lobules which are the seat of prior necrosis. From the findings of the present series it is impossible to state whether or not lobular disruption can occur as a purely autolytic phenomenon, though it had not occurred 96 hours after autolysis. From the above observations it is apparent that postmortem changes will tend to augment the histological picture of a wide variety of hepatic lesions such as necrosis, hepatitis, chronic passive congestion, and the like, making accurate assessment of structural premortem changes difficult.

SUMMARY

The histological features of postmortem autolysis under nonsterile, room-temperature conditions have been studied and compared in the kidney of the rabbit, rat, and man and in the liver of the rat and man. The human tissues were autolyzed in saline-moistened toweling; the animal tissues were immersed in isotonic saline or left in situ in the carcass.

The autolytic picture in the proximal convoluted tubules differs from that in the remainder of the nephron. In the former site the process is one of cytokaryolysis, while in the latter site acidophilic density of the cytoplasm occurs along with pyknosis of the nuclei.

The influence of conditions of storage produces only minor qualitative changes in the autolytic process, related to fluid and bacterial action. The quantitative effect is marked.

It was concluded that antemortem degenerative changes of the kidney are character-

ized in the following way. Degenerative changes in the proximal convoluted tubules are readily distinguishable from the changes of postmortem autolysis by the presence of nuclear pyknosis and increased cytoplasmic staining intensity. In the distal convoluted tubules, the presence of patchy areas of nuclear pyknosis, acidophilia, and increased density of cytoplasm represents premortem changes. These diagnostic features are most pronounced with the use of Masson's trichrome stain. They are lost in the late stage of autolysis with the disappearance of nuclear and cellular structure.

In the rat liver, during the first 20 hours of autolysis there is noted progressively increased pallor of staining in the cytoplasm and nucleus of the parenchymal cell and a slight shrinkage in volume. After 44 hours, however, the cord cells assume an increased intensity of stain in both cytoplasm and nucleus. The nuclear changes may subsequently progress to pyknosis and karyorrhexis. The biphasic nature of the changes in the hepatic cord cell would appear to be related to the glycogen content of the cell. The changes in human liver tissue are similar in the majority of specimens.

A portion of this investigation was performed under the supervision of Prof. A. M. Drennan, at the University of Edinburgh. Prof. Drennan gave advice and criticism, and Mr. T. C. Dodds supplied all photographs.

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Glomerulotubular Nephrosis Correlated with Hepatic Lesions

II. Incidence and Morphology of Associated Kidney and Liver Lesions in Human Autopsy Material

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Since Bywaters' description of the "crush syndrome" and acute renal failure, there has been much interest in the kidneys of acute renal failure which accompany a broad spectrum of clinical entities.* A select group of cases in which acute oliguria or anuria accompanies vaguely defined hepatic and renal damage has been referred to as the "hepatorenal syndrome." In addition, there are reports of infection, chemical poisons, allergies, etc., leading to death from renal failure in cases with associated lesions of both kidneys and liver. Some recent autopsy cases revealed damage in both livers and kidneys in the absence of the clinical picture of the hepatorenal syndrome. In view of this, it seemed desirable to ascertain whether any specific pathological process in the kidneys could be found in cases with varying types of hepatic disorders and to determine the exact nature of the renal lesion, when present. The following study of postmortem material demonstrated the regular association of a lesion in the kidney, which we have designated as "glomerulotubular nephrosis," with a wide variety of lesions of the liver.

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Medical Research Fellow, National Research Council of Canada (Dr. Crowson).

From the Departments of Pathology, Queen's University and the Kingston General Hospital, Kingston, Ont., Canada, and the University of Edinburgh, Scotland.

* References 1 through 9.

MATERIALS AND METHODS

Cases with associated hepatic and renal lesions were obtained from the autopsy service of the Kingston General Hospital and chosen from two separate periods. The current autopsy material during the period from July 1, 1951, to May 31, 1952, was reviewed in its entirety, cases being chosen from those with renal or hepatic alterations or both. In addition, the autopsy files were studied from the year 1948, cases being selected from entries on the final anatomical diagnoses which implicated the kidneys, the liver, or both. The entire sampling group equaled approximately 400 autopsies.

After this selection there remained 50 cases with nonspecific glomerular and tubular damage. The renal lesions of the selected cases of nonspecific combined glomerular and tubular damage included 5 cases of "lower nephron nephrosis" (diagnosed on clinical and morphological grounds), 8 cases of nephrosis of varying etiology (e. g., bile, toxic, etc.), and 37 cases of "early nonspecific nephrosis." On the basis of this selection there was associated liver pathology in every case of glomerulotubular nephrosis and vice versa.

The final selection from those cases reviewed was made on the basis of the renal lesion. This was done because the end-point in this study was a renal glomerulotubular lesion which appeared to represent a morphological entity. Therefore, all cases of renal disease such as glomerulonephritis, pyelonephritis, nephrolithiasis, obstructive uropathy, arteriolonephrosclerosis, marked arteriosclerotic scarring, and polycystic and other developmental defects and neoplasm were excluded. In addition, postmortem autolysis, when of sufficient severity to obscure antemortem change, was used as a ground for rejection.[10]

To control the selected material, it was necessary to determine whether renal lesions of the type present in the above group ever occur in the absence of hepatic disorders. It was also imperative to be able

TABLE 1.—*Associated Clinical and Pathological Findings in Fifty Cases of Glomerulotubular Nephrosis*

Case	Primary Disease	Associated Diseases	Hepatic Lesion	Recorded Additional Renal Lesions*	Wt. of Kidneys, Gm.	Wt. of Liver, Gm.	Age, Yr.†	Sex	Hours Post Mortem
1	Acute encephalitis	.....	Fatty metamorphosis and C. P. C.‡	Nil	320	2050	22	F	1½
2	Appendicitis; postoperative shock and peritonitis	Acute gangrenous appendicitis	Focal necrosis; fatty metamorphosis	Nil	350	1950	52	M	6
3	Carcinoma of rectum	Pulmonary edema; bronchopneumonia	Centrolobular atrophy and necrosis	Moderate arteriosclerosis	425	1600	69	M	2
4	Burns; shock	Acute bronchopneumonia	Centrolobular necrosis	Lower nephron nephrosis	450	2150	47	M	?
5	Subdiaphragmatic abscess	Acute diffuse peritonitis	Acute edema and focal necrosis	Lower nephron nephrosis	300	2100	55	M	9
6	Carcinoma of large intestine	Secondary carcinoma of brain	Extreme C. P. C.	Nil	300	1550	45	F	6½
7	Hypertensive and coronary heart disease	Acute bronchopneumonia	Centrolobular necrosis and marked C. P. C.	Nil	365	1625	70	M	8
8	Carcinoma of pancreas	Biliary cirrhosis	Biliary cirrhosis	Bile nephrosis of kidney	275	1300	74	F	1
9	Massive pulmonary embolism	Generalized arteriosclerosis	Severe C. P. C.	Nil	180	1125	92	M	3½
10	Hypertensive heart disease	Myocardial infarction	Severe C. P. C.	Nil	275	1350	50	M	?
11	Carcinoma of large intestine	Acute diffuse peritonitis	Severe C. P. C. and centrolobular necrosis	Nil	255	1650	69	M	?
12	Coronary heart disease	Arteriosclerosis of coronary arteries	Severe C. P. C.	Nil	230	1500	64	M	2
13	Acute pancreatitis	Peritonitis	Cirrhosis, fatty metamorphosis and C. P. C.	Nil	335	2150	26	M	2½
14	Fracture of skull; edema of brain	Portal cirrhosis	Cirrhosis, fatty metamorphosis and focal necrosis	Nil	355	2325	33	M	4
15	Carcinoma of thyroid	Secondary carcinoma of lungs, lymph node, superior vena cava	Moderate C. P. C.	Nil	120	1080	66	M	?
16	Addison's disease	T. B. of adrenals (bilateral)	Focal necrosis and edema	Misdiagnosed as cortical necrosis	225	1225	54	F	8
17	Rheumatic heart disease	Severe mitral stenosis	Severe C. P. C. and centrolobular necrosis	Nil	410	1800	48	M	½
18	Chronic myelogenous leukemia	Thrombosis of pulmonary artery	Leukemia, infiltration, and moderate C. P. C.	Nil	300	2500	27	M	¾
19	Cholodocholithiasis; biliary cirrhosis	Ruptured esophageal varices (terminal)	Biliary cirrhosis and focal necrosis	Nil	375	1700	47	F	6½
20	Typhoid fever	.....	Focal necrosis; cholangiohepatitis	Nil	438	2160	15	M	5
21	Adenocarcinoma of colon	Subdiaphragmatic abscess	Centrolobular necrosis and atrophy	Nil	325	2000	68	M	10

TABLE 1.—*Associated Clinical and Pathological Findings in Fifty Cases of Glomerulotubular Nephrosis*—Continued

Case	Primary Disease	Associated Diseases	Hepatic Lesion	Recorded Additional Renal Lesions*	Wt. of Kidneys, Gm.	Wt. of Liver, Gm.	Age, Yr.†	Sex	Hours Post Mortem
22	Portal cirrhosis	Ruptured esophageal varices (terminal)	Cirrhosis and focal necrosis; acute hepatitis	Nil	250	1050	41	F	6½
23	Congenital malformation of heart	Acute myocardial failure	Severe C. P. C. and centro-lobular necrosis	Nil	275	1250	36	F	1
24	Idiopathic hypertrophy of heart	Myocardial failure	Severe C. P. C. and fatty meta-morphosis	Nil	62.5	250	6 mo.	F	3
25	Carcinoma of gall bladder	Biliary cirrhosis (early)	Tumor and cirrhosis	Bile nephrosis	250	2850	77	F	10
26	Duodenal ulcer	Portal cirrhosis of liver	Cirrhosis and focal necrosis	Nil	425	2200	59	F	1¾
27	Congenital heart disease	.....	Centro-lobular necrosis	Cortical necrosis (minimal)	20	85.5	2 days	F	2
28	Reticulum cell sarcoma	Anemia (Hgb 5.6 gm.)	C. P. C.	Nil	250	1350	72	M	1
29	Alcoholism; portal cirrhosis	Hemorrhag-ing peptic ulcer	Cirrhosis and focal necrosis	Nil	450	1700	60	M	12
30	Peptic ulcer; gastrectomy	Massive atelectasis	Centro-lobular necrosis	Nil	350	2200	49	M	8
31	Pernicious anemia	Acute yellow atrophy of liver	Massive hepatic necrosis and bile retention	Bile nephrosis	300	850	52	F	½
32	Carcinoma of stomach	Carcinoma of prostate	Tumor and fatty meta-morphosis	Arterio-sclerotic scarring (slight)	325	1700	87	M	5
33	Subdural hematoma	Alcoholism	Cirrhosis and fatty metamor-phosis	Arterio-sclerotic scarring (slight)	350	2100	66	M	13
34	Perforation of ileum	Peritonitis	Cloudy swelling	Arterio-sclerotic scarring (slight)	202	1200	73	M	12
35	Thymoma	Cirrhosis of liver	Cirrhosis and C. P. C.	Nil	250	1000	47	M	6
36	Acute broncho-pneumonia	Acute nephrosis and cerebral thrombosis	Acute edema and C. P. C.	Regenerating nephrosis	30	100	5 wk.	M	11
37	Peptic ulcer; gastrectomy	Bile peritonitis	Focal necrosis; acute edema and fatty metamor-phosis	Lower nephron nephrosis	400	1800	49	M	½
38	Alcoholism	Portal cirrhosis	Cirrhosis	Arterio-sclerotic scarring (slight)	...	....	65	M	12
39	Alcoholism	Acute nephrosis	Edema and fatty meta-morphosis	Acute nephrosis	440	2550	30	M	10
40	Lutem-bacher's disease	Cardiac cirrhosis	Cirrhosis and centro-lobular necrosis	C. P. C.	300	1400	44	F	1
41	Embolism of femoral artery	Involutional melancholia	Centro-lobular necrosis	Arterio-sclerotic scarring (slight)	170	750	67	F	3
42	Coronary heart disease	Diabetes	Centro-lobular necrosis and atrophy	Nil	440	1800	57	F	8

TABLE 1.—*Associated Clinical and Pathological Findings in Fifty Cases of Glomerulotubular Nephrosis*—Continued

Case	Primary Disease	Associated Diseases	Hepatic Lesion	Recorded Additional Renal Lesions*	Wt. of Kidneys, Gm.	Wt. of Liver, Gm.	Age, Yr.†	Sex	Hours Post Mortem
43	Pulmonary fibrosis; healed T. B.	Cor pulmonale	Centrolobular atrophy, necrosis, and fibrosis	Nil	400	1250	47	M	4
44	Coronary thrombosis	Myocardial failure	Edema and C. P. C.	Nil	400	2000	65	M	1½
45	Pulmonary T. B.	Cachexia	Early cirrhosis, focal necrosis, and C. P. C.	Nil	250	1050	31	F	10
46	Carcinoma of breast	Hepatic coma	Atrophy and marked secondary tumor	"Bile nephrosis"	350	4700	52	F	12
47	Hepatic centrolobular necrosis	Toxic (?)	Centrolobular necrosis, confluent	? Toxic nephrosis	190	350	13 mo.	M	6
48	Pulmonary fibrosis and cor pulmonale	Cardiac cirrhosis	Centrolobular fibrosis, necrosis, atrophy, and congestion	Nil	350	1175	48	M	4
49	Carcinoma of the head of the pancreas; Roux-Y	Gangrene of small bowel	Centrolobular necrosis; bile stasis	Slight arteriosclerosis	320	1200	67	M	7
50	Trauma; skull fracture	Intracerebral hemorrhage	Centrolobular necrosis	Lower nephron nephrosis	395	2050	19	M	10

* Renal lesions which were recorded on the autopsy protocol.
† Age in years, except as otherwise designated.
‡ Chronic passive congestion.

to assess the effect of early autolytic changes on both normal and abnormal kidneys, as reported in the preliminary paper.[10] The 15 cases constituting the controls were gleaned from the files of the following institutions: Kingston General Hospital, 3; Kingston Hotel Dieu Hospital, 1; Toronto Medico-Legal Department,† 1; Edinburgh Royal Hospital for Sick Children, 5; other regional hospitals of the southeast area, 5. (The presence of refrigeration facilities for the control group is noted in Table 2). In all instances, paraffin sections were cut at 5μ and stained with hematoxylin and eosin and Masson's trichrome stain. Ten per cent formalin was employed as the routine fixative throughout this study. The control and selected material was then studied with reference to the exact morphologic characteristics of the renal lesion and the correlation of this with alterations in the liver.

RESULTS

General Clinical and Pathological Findings.—The clinical and pathological findings and correlations of the selected and control groups are presented in Tables 1 and 2, respectively.

The following assessment of the incidence of clinical diseases accompanying the hepatic and renal lesions is derived from the "primary disease" and "associated diseases" columns of Table 1. It will be readily appreciated that these figures include several cases with more than one clinical disease, which alters the values for relative incidence. Cirrhosis of the liver, congestive heart failure, and malignant tumors each accounted for 26% of the total, the latter being present obstructively in the biliary tract in only 8% of cases. Peritonitis was found in 14%, and bronchopneumonia, alcoholism, and cerebral damage each accounted for 8%. The remainder was composed of a wide variety of entities, e. g., burns, rupture of esophageal varices, duodenal ulcer and its complications, pulmonary tuberculosis, Addison's disease, acute pancreatitis, infectious hepatitis, major abdominal surgery, etc. Obviously, few of these entities

† This case was obtained through the courtesy of Dr. C. R. Maclean.

TABLE 2.—*Control Series (Fifteen Cases) from Kingston, Toronto, and Edinburgh, Since 1948*

Case	Primary Disease	Associated Diseases	Hepatic Lesion	Recorded Additional Renal Lesion*	Wt. of Kidneys	Wt. of Liver	Age	Sex	Hours Post Mortem	Refrigeration
1	Traumatic aortic rupture	.....	Slight terminal congestion	Nil	.....	.....	15	M	4	+
2	Pulmonary tuberculosis	Thoracentesis and sudden death	Slight centrolobular atrophy	.....	.....	.....	36	M	5	+
3	Cerebral hemorrhage	.....	Slight fatty metamorphosis	Moderate arteriosclerosis	.....	.....	72	F	10	+
4	Congenital heart disease	.....	Nil	Medullary hyperemia	....	.....	S. B.	F	12	−
5	Fractured skull	Extradural hemorrhage	Slight fatty metamorphosis	Nil	. ...	.....	37	M	10	+
6	Traumatic death	.....	Nil	Nil	Avg.	Avg.	4	F	24	−
7	Bacillary dysentery	(Sonne)	Slight terminal congestion	Nil	Avg.	Avg.	4	F	4	−
8	Virus pneumonia	.....	Slight terminal congestion	Nil	Avg.	Avg.	2½	M	8	−
9	Severe scalds	Acute heart failure	Terminal acute congestion	Nil	Avg.	Avg.	3	F	24	−
10	Virus pneumonia	.....	Slight terminal congestion	Nil	Avg.	Avg.	2	F	12	−
11	Traumatic death within 2 hr.	.....	Moderate autolysis	Moderate autolysis	Avg.	Avg.	41	M	48	+
12	Postoperative peritoneal adhesions	Intestinal obstruction	Moderately advanced autolysis	Moderately advanced autolysis	Avg.	Avg.	48	F	72	−
13	Acute rheumatic fever	Acute cardiac failure	Terminal acute congestion	Acute congestion	Avg.	Slightly enlarged	3½	M	24	−
14	Traumatic death	Rupture of spleen and lungs; intracranial hemorrhage	Nil	Nil	Avg.	Avg.	17	M	24	−
15	Femoral phlebothrombosis	Massive pulmonary embolus	Terminal acute congestion	Terminal congestion	Avg.	Avg.	34	F	5	−

* Renal lesions which were recorded on the autopsy protocol.

would fit the "classical" picture of the hepatorenal syndrome, vaguely defined as "hepatic necrosis plus acute renal failure related pathogenetically to surgical (and traumatic) intervention in the biliary and gastrointestinal tracts, liver, and thyroid." [11]

At autopsy, there were no constant gross characteristic features in the kidneys. The average combined weight of these organs was well within normal limits, with 10 (22% of adults) weighing over 400 gm. and 6 (13% of adults) weighing under 250 gm. On the other hand, most livers showed some abnormal macroscopic feature, e. g., cirrhosis, massive necrosis, severe chronic passive congestion, tumor, etc. The majority of the livers (30, or 66% of adults) were increased in weight above 1400 gm., while 6 (13% of adults) were below 1200 gm. These figures relate only to the adult segment of the selected population, which comprised 92% of the total. The ages ranged from 2 days to 92 years, the mode being 50 years.

The histopathology of the liver is recorded in Table 1. Chronic passive congestion of the liver was a feature of 44% of the total and was coexistent with shock in some cases. Necrosis of the liver occurred in 56% of cases, distributed centrolobularly in 32%, focally in 22%, and massively in one case

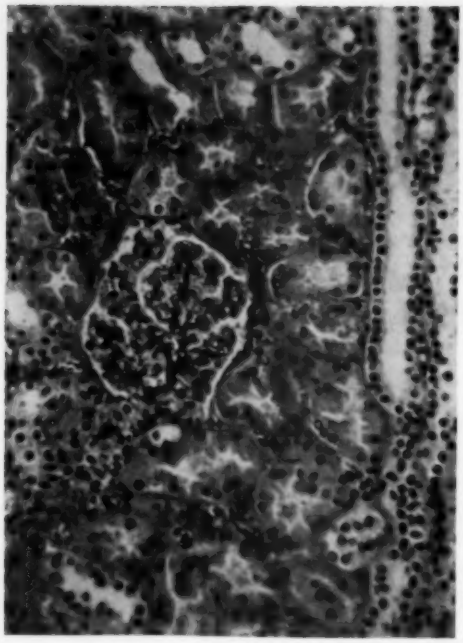


Fig. 1 (Case 11).—Control. Normal kidney. Hematoxylin and eosin; mag. × 200.

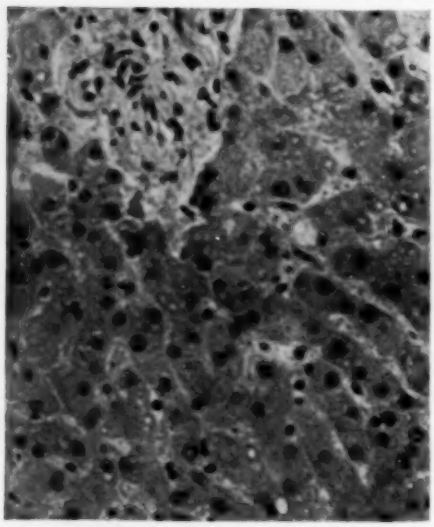


Fig. 2 (Case 11).—Control. Normal liver showing a very slight degree of fatty change. Hematoxylin and eosin; mag. × 300.

TABLE 3.—*Lesions of Glomerulotubular Nephrosis* *

Renal Lesions	Lower Nephron Nephrosis, 5 Cases		Bile, Toxic, etc., Nephrosis, 8 Cases		Early Nonspecific† Nephrosis, 37 Cases		Total		Controls	
	No.	Per Cent	No.	Per Cent	No.	Per Cent	No.	Per Cent	No.	Per Cent
1. Glomerulus										
(*a*) Exudate into capsular space	4	80	7	88	36	97	47	94	8	54
(*b*) Swelling of capsular epithelium	5	100	8	100	34	92	47	94	3	20
(*c*) Increased cellularity of tuft	0	0	2	25	3	8	5	10	0	0
(*d*) Epithelial desquamation	5	100	8	100	37	100	50	100	6	40
(*e*) Epithelial crescents	0	0	0	0	0	0	0	0	0	0
2. Proximal tubules										
(*a*) Dilatation of lumina	4	80	6	75	31	84	41	82	1	7
(*b*) Pyknosis of nuclei	5	100	5	63.5	37	100	47	94	3	20
(*c*) Coagulation of cytoplasm	5	100	7	88	36	97	48	96	1	7
(*d*) Cellular and amorphous casts	5	100	7	88	37	100	49	98	1	7
(*e*) Epithelial desquamation	3	60	5	63	35	95	43	86	1	7
3. Thin limb of loop of Henle										
(*a*) Hyaline casts	5	100	8	100	37	100	50	100	4	27
4. Distal tubules (including thick limb of Henle)										
(*a*) Pyknosis of nuclei	5	100	8	100	34	92	47	94	8	54
(*b*) Coagulation of cytoplasm	4	80	7	88	29	78	40	80	3	20
(*c*) Dilatation of lumina	5	100	8	100	34	92	47	94	4	27
(*d*) Cellular casts	5	100	8	100	35	95	48	96	1	7
(*e*) Hyaline casts	5	100	7	88	37	100	49	98	4	27
(*f*) Granular casts	5	100	8	100	27	77	40	80	4	27
(*g*) Pigmented casts										
(*i*) Bile	0	0	4	50	0	0	4	8	0	0
(*ii*) Hyalo- and/or granular-heme	5	100	1	13	20	54	26	52	2	13
(*h*) Epithelial desquamation	4	80	5	63	26	70	35	70	8	54
(*i*) Fatty vacuolation	1	20	4	50	13	35	18	36	0	0
(*j*) Tubal rupture	3	60	1	13	0	0	4	8	0	0
(*k*) Regeneration	2	40	1	13	0	0	3	6	0	0
5. Collecting tubules										
(*a*) Casts	5	100	7	87.5	36	97	48	96	1	7
6. Interstitium										
(*a*) Edema	5	100	6	75	25	68	36	72	1	7
(*b*) Inflammatory cell infiltration	4	80	4	50	8	22	16	32	0	0
(*c*) Granulomatous reaction	4	80	2	25	1	3	7	14	0	0
7. Blood vessels										
(*a*) Angiitis	3	60	1	13	1	3	5	10	0	0
(*b*) Cortical collapse and medullary hyperemia	2	40	1	13	0	0	3	6	0	0

* An analysis of 50 cases from the Kingston General Hospital autopsy files in the years 1948 and 1951-1952, and of 15 control cases from Kingston, Toronto, and Edinburgh.

† See text, Materials and Methods, for term "early nonspecific nephrosis."

(2%). Other hepatic lesions included severe fatty metamorphosis in 18%, bile retention in 12%, diffuse fibrosis (cirrhosis) in 26%, plus other disturbances of minor incidence: acute edema, cholangiohepatitis, leukemic infiltration, "cloudy swelling," and metastatic tumor.

Renal lesions of the selected group designated "glomerulotubular nephrosis" consisted of degenerative changes in the glomerulus and all parts of the nephron. The detailed morphological features of this lesion will be described below. In addition, under the classification "additional renal disease" in Tables 1 and 2, lesions other than glomerulotubular nephrosis, e. g., mild arteriosclerosis, bile nephrosis, etc., are recorded directly from the autopsy protocol.

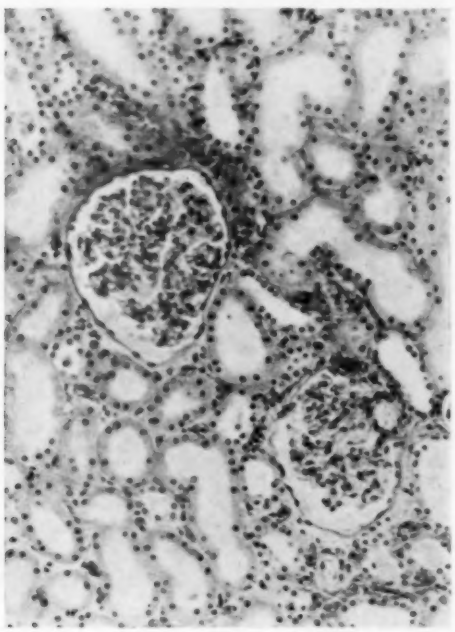


Fig. 3 (Case 20).—Kidney in typhoid. Marked dilatation of proximal tubules, with protein and cellular material in lumina and capsular spaces and widespread nuclear pyknosis. The thin limbs of Henle's loop contain hyaline casts. Hematoxylin and eosin; mag. × 150.

In the control series, minor antemortem changes occurred in 67% of livers and 27% of kidneys and include terminal hyperemia and minimal fatty metamorphosis of the

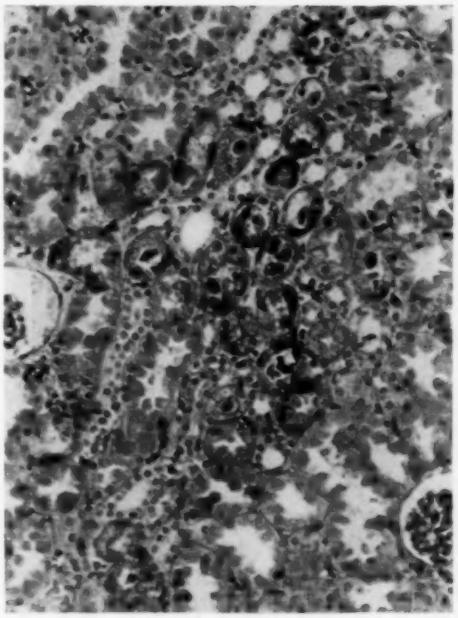
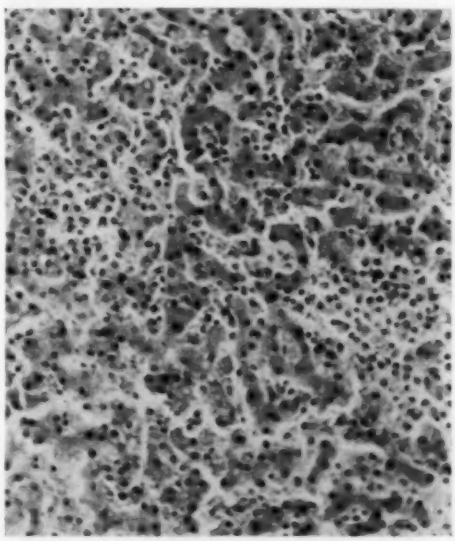


Fig. 4 (A-147-49).—Unlisted. Kidney from another case of typhoid. The acute focal lesions situated in the proximal and distal tubules stand out with this stain. Exudate is present in capsular space. Trichrome stain; mag. × 150.

Fig. 5 (Case 20).—Liver from the case of typhoid shown in Figure 3, displaying the characteristic focal necrosis. Hematoxylin and eosin; mag. × 150.



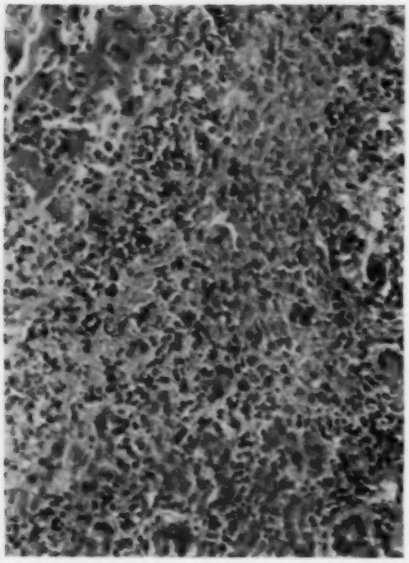
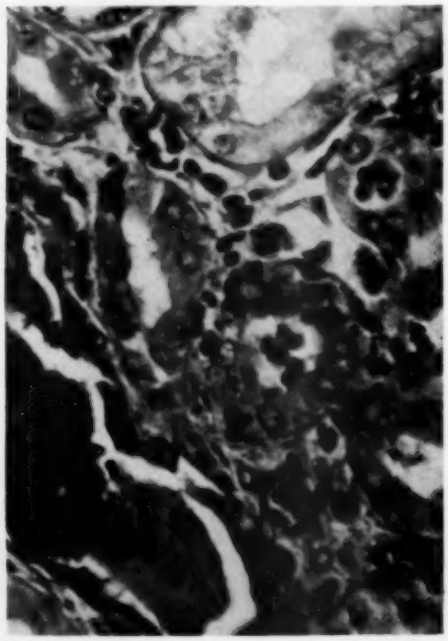


Fig. 6 (Case 31).—Liver from a case of massive hepatic necrosis, showing extensive parenchymal collapse and acute necrosis. Trichrome stain; mag. × 150.

Fig. 7 (Case 31).—Kidney from same case as in Figure 6, displaying the acute lesion in proximal tubules of cytonuclear degeneration and sloughing, and the chronic atrophic changes of the epithelium of the distal tubules in the region of "foreign body" bile pigment casts. Note the large vacuolar cells of hydropic degeneration in a proximal segment. Trichrome stain; mag. × 150.



liver and hyperemia of the kidney. In the kidney, the total picture of degenerative changes could not be interpreted as glomerulotubular nephrosis. Figures 1 and 2 are examples of normal kidney and liver, respectively, from Case 11, Table 2.

Microscopic Characteristics of Glomerulotubular Nephrosis.—From Table 3 the following lesion in the nephron may be constructed.

Glomerular changes are those of protein exudate and capsular epithelial desquama-

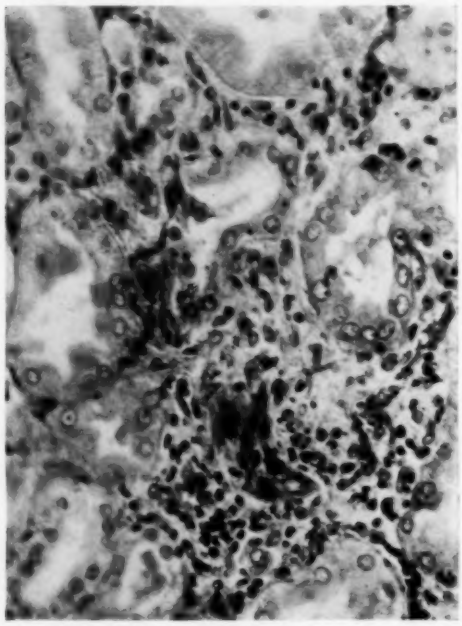


Fig. 8 (Case 31).—Kidney from same case as in Figures 6 and 7, showing the typical interstitial changes of the so-called "lower nephron nephrosis," usually interpreted as the result of tubal rupture and extrusion of protein, etc., casts. Trichrome stain; mag. × 300.

tion in the majority of capsular spaces, plus varying degrees of swelling of the capsular epithelial cells. (Exudate and epithelial desquamation were also found, albeit in far slighter degree, in roughly 50% of the control kidneys.) Most of these features are well displayed in Figures 3, 4, and 10.

Proximal convoluted tubular changes are those of slight to moderate luminal dilatation, plus varying degrees of acute degenera-

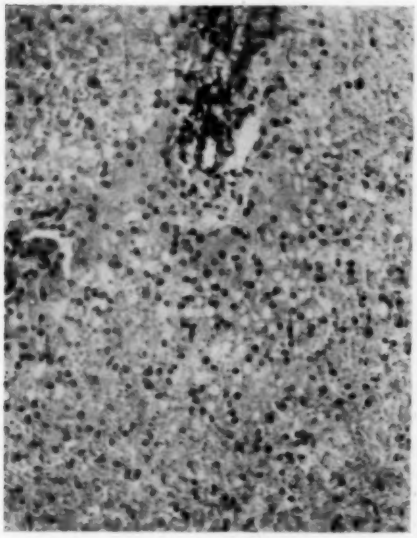


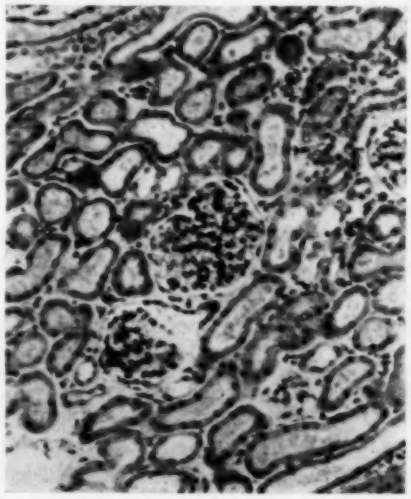
Fig. 9 (Case 47).—Liver from case of confluent centrolobular necrosis of unknown (? toxic) etiology. Surviving liver tissue show up as narrow bands in wide fields of hemorrhage and necrosis. Hematoxylin and eosin; mag. × 150.

tion of the epithelial cells from nuclear pyknosis and "coagulative" hyperchromaticity (bright, smudgy, or more rarely corpuscular, red with the trichrome stain) of the cytoplasm to shedding of the necrotic cells with the formation of mixed cellular and amorphous casts. (The latter are usually numerically small but almost invariably present.) All changes show a patchy distribution, usually more pronounced in the boundary zone between cortex and medulla. The presence of bile tends to decrease the nuclear staining intensity to a moderate degree. The changes in the proximal tubules are well illustrated in Figures 3 and 4. (The hepatic lesion accompanying this case is shown in Figure 5.)

Descending limb of Henle's loop shows changes reflecting the increased permeability of the glomerular filter, namely, hyaline casts, composed of inspissated protein material. (These casts stain a pale green with Masson's trichrome stain.)

Distal convoluted tubular changes (including the ascending limb of Henle's loop) are those of luminal dilatation, the tubules containing a wide variety of formed casts, including cellular, granular, hyaline, and pigmented types, with atrophic thinning of the epithelial lining surrounding the larger casts. There is also a patchy acute degeneration of the epithelium characterized by coagulation and eosinophilia of cytoplasm, simple nuclear pyknosis, and epithelial sloughing. This is indistinguishable from postmortem autolysis and represents premortem change only when patchy areas are clearly in advance of the remainder of the distal convoluted tubules.[10] (The presence of a few large, hyaline casts, usually containing some orange-brown pigment, is a "normal" feature of the adult human kidney, but if present in large numbers, especially if mixed with granular pigmented casts, they may signify glomerulotubular nephrosis in its more protracted forms.) Fatty vacuolation is present in 50% of the selected group when the case protocol defines an etiologic toxic factor, but it is negligible in the other varieties. Similarly, bile pigment is found only in the cholemic type of nephrosis. Tubal rupture and regeneration is seen primarily in the cases diagnosed on the protocol as "lower nephron nephrosis" and is also noted in the case of massive hepatic necrosis. (The

Fig. 10 (Case 47).—Kidney from case illustrated in Figure 9. Note changes which are practically identical to those in Figure 3. There is moderate swelling of the capsular epithelium. Hematoxylin and eosin; mag. × 150.



accompanying interstitial reaction is illustrated in Figure 8.)

Interstitial changes are primarily those of edema. Inflammatory reactions are seen only in the more protracted lesions.

Vascular changes are again rather specific to the protracted forms of nephrosis, angiitis being present in 60% of cases of "lower nephron nephrosis" and in the case of cholemic nephrosis associated with massive hepatic necrosis. Cortical ischemia and medullary hyperemia show a rather similar distribution and incidence.

Figure 6 depicts the liver from the case of massive hepatic necrosis, and Figures 7 and 8 show the kidney from the same case. The changes described in the distal portion of the nephron are apparent in these last two. Figures 9 and 10 depict, respectively, the liver and kidney lesions in a case of confluent centrolobular necrosis of the liver, etiology unknown.

COMMENT

The significant fact established from this study is the regular association between liver damage of varying types and a degenerative glomerular and tubular lesion exhibiting rather constant features. The renal lesion found in the selected group was similar in quality to the nephrotoxic and tubulorrhectic lesions that Oliver and co-workers [8] described so well in cases of acute renal failure. However, except for those cases of obvious severe renal damage diagnosed as "lower nephron nephrosis" in the autopsy protocols, the changes were, by comparison, minimal. It has been decided to name this lesion "glomerulotubular nephrosis," but the terms "nephrotoxic necrosis" and "tubulorrhexis" of Oliver, and "glomerulonephrosis" of French,[7] appear to be designations for the same pathologic process. It is obvious that no term that has either a pathogenetic or etiologic connotation can, as yet, be applied to these lesions. Since terms like "lower nephron nephrosis" conjure up a picture of acute renal failure and are anatomically incorrect, it would be preferable to use another anatomical term to describe the renal lesion that appears to bear such a constant association to structural hepatic disorders often of minor degree and varying type.

The exact significance of the relationship between the hepatic and renal lesions cannot be discerned from the findings in this study alone. However, the facts suggest that morphologic damage with resultant dysfunction of the liver may be a preceding cause in the chain of events leading to the renal lesion. This possibility is supported by the constancy of the type of renal change and the wide variability of the inevitably associated structural changes in the liver. However, assuming that liver damage precedes and in some way causes glomerulotubular nephrosis, it might be argued that the disorder in many of the livers was too minimal to produce hepatic dysfunction. The relation between pathologic histology and physiology cannot be correlated accurately as yet [12]; but since dysfunction may be present without demonstrable morphologic alterations in the liver, it is reasonable to believe that the minimal lesions seen in many of the livers could be associated with significant liver dysfunction.

In any case, renal damage secondary to various forms of liver disease has been reported quite frequently in the medical literature, viz.; Fahr [13] described "bile nephrosis" in 1925; Furtwaengler [14] reported the first case of hepatorenal syndrome in 1927; Heintzelmann [15] and Farquhar [16] have recorded abnormalities of renal function in cases of infectious hepatitis; Baxter and Ashworth,[17] Epstein and co-workers,[18] and Patek and co-workers [19] have shown an interesting correlation of cirrhosis of the liver, impaired renal function, and chronic "intercapillary" glomerulonephritis, while Morrison [20] reported improved renal function in cases of nephrosis plus cirrhosis following dietary treatment of the cirrhosis; French [7] noted that the state of the liver was related to "glomerulonephrosis."

Tomb,[21] in 1942, postulated an anoxic basis as the intrarenal mechanism for the various clinical forms of acute renal failure, and, subsequently, the ischemic etiology of

the lesions of the anuric kidney has been widely accepted.‡ The features of glomerulotubular nephrosis strongly suggest focal ischemia as a potential factor in its pathogenesis. When the correlated hepatic changes are considered, the interesting possibility arises that circulating vasopressor substances, normally removed by the liver, tend to accumulate during a period of hepatic insufficiency and produce an acute ischemic state in the kidneys. Such a view provides one explanation for the curious and unexplained diuresis seen suddenly around the 10th to the 14th day in cases with acute renal failure, suggesting that the resurgence of renal function might depend upon the adequate resumption of the process of detoxification by the liver. Despite its plausibility, it is impossible to prove the above hypothesis on the basis of the material in this study, and a few alternative hypotheses regarding the pathogenesis should be mentioned.

Earlier investigators of the hepatorenal syndrome § favored the view that necrotic liver cells released a nephrotoxin into the circulation, or that, alternatively, hepatic necrosis caused failure of detoxification of a toxin formed in the gut, which thus passed directly into the systemic circulation. The former view is not in accord with the observation that 50% of the present cases had no necrosis in the liver. French [7] has suggested hyperglobulinosis in chronic liver disease as a possible etiologic link in "glomerulonephrosis." However, chronicity is absent in a large percentage of our cases, arguing against this postulate. An alternative explanation of pathogenesis may be that the same ischemic or toxic agent acts on liver and kidney simultaneously. In the present study, shock (a potential ischemic factor to both liver and kidney) was present in a number of cases, but the exact incidence could not be determined from the clinical protocols.

In the present study, the facts give strong support to the concept of a hepatic causation of the renal lesion. If this proves to be correct, it follows that the present group of cases are early manifestations of something quite akin to the hepatorenal syndrome. Since this syndrome is already vague and ill-defined, it seems appropriate to enlarge the concept to include the findings of the present study.

‡ References 8, 9, 22, and 23.

§ References 14, 24, and 25.

SUMMARY

Glomerulotubular nephrosis, the basic histological pattern of acute degenerative lesions in the nephron, is described in detail.

A close correlation is found to exist between glomerulotubular nephrosis and structural changes in the liver.

The hepatic changes consist of acute and chronic lesions and vary from severe fatty metamorphosis, cirrhosis, and acute edema, through all stages of atrophy and minor necrosis to massive hepatic necrosis.

The hypothesis is proposed that vasopressor materials accumulate in the systemic circulation during periods of hepatic insufficiency and produce renal ischemia.

The correlation is felt to comprise part of the "hepatorenal syndrome," and it is recommended that the syndrome, already nebulous and ill-defined, be extended to include these related phenomena.

Prof. A. M. Drennan gave valuable criticism and advice. Photomicrographs of the lesions were supplied by Mr. P. H. Mott, of Queen's University, and the controls by Mr. T. C. Dodds, of the University of Edinburgh.

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Glomerulotubular Nephrosis Correlated with Hepatic Lesions

III. Production of Acute Glomerulotubular Nephrosis in the Rabbit by Means of Hepatic Surgery

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Preliminary papers in this series * have dealt with the natural incidence of hepatic lesions and coexistent glomerulotubular nephrosis (hereinafter referred to as G. T. N.) in human autopsy material and their differentiation from autolytic tissue changes. G. T. N. was shown to consist of focal degenerative changes in glomeruli and tubules. The present investigation was undertaken in an attempt to study the influence of acute experimental liver damage on the genesis of G. T. N. lesions in the kidney of the rabbit. Helwig and Schutz [3] reported hepatic and renal necrosis in dogs following experimental occlusion of the hepatic artery or traumatic rupture of the liver, in 1932. In 1935, Boyce and McFetridge [4] were unsuccessful in producing renal lesions in rabbits by similar procedures, though they succeeded in doing so through the relief of temporary (2-week) biliary obstruction. The following year, Pytel,[5] using the techniques of Helwig and Schutz,[3] succeeded in the experimental production of the hepatorenal syndrome in rabbits. In view of these conflicting reports it was decided to approach the investigation along slightly different lines, avoiding, where possible, such complications as shock, cholemia, and chronicity. The object of the experiments was to produce a state of acute, uncomplicated, hepatic damage in rabbits and to assess the influence of such a condition upon the otherwise normal kidneys.

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* References 1 and 2.

MATERIALS AND METHODS

Forty-nine young (1300 to 2500 gm.) rabbits of both sexes were utilized. An exploratory pilot experiment was devised to determine the most satisfactory approach, using 11 rabbits. The operative procedures employed included ligation of either the porta hepatis, the portal vein, or the hepatic artery in the free zone of the porta (total of five rabbits) and temporary occlusion of the porta hepatis (six rabbits). After this preliminary study, further investigation was conducted along the following lines: (*a*) temporary occlusion of the porta hepatis in 12 rabbits, porta clamped off for periods up to 30 minutes as conditioned by the clinical status; (*b*) unoperated controls (17 rabbits); (*c*) simple laparotomy controls (9 rabbits).

All operations were carried out under open-ether anesthesia. Through a 3-inch (7.6 cm.) median incision the porta hepatis was cleared as gently as possible and the clamp applied. The latter consisted of Willets forceps whose blades were encased in firm gum rubber tubing, so that the rubber surfaces were in gentle apposition. The clamp was allowed to rest against the rib margin, and the incision was sutured with cotton in two layers, from the lower end of the incision to within 1 inch (2.5 cm.) of the portal clamp. Ether was given as required, and the clamp was left in position for as long an interval as possible up to a maximum of 30 minutes. (Usually between 15 and 20 minutes, to avoid shock.) In the two laparotomy control animals of Group F, the left renal pedicles were occluded for 10-minute periods.

The animals were usually conscious within a few minutes after cessation of the operation. Postoperatively all animals were placed in metabolism cages and the urine output recorded. Urine for urinalysis was obtained at time of autopsy as a bladder specimen by means of a syringe and needle. Animals which died during the operation were brought to autopsy immediately. The remainder were killed by air embolism approximately 24 hours postoperatively. One animal, A-6, died 8 hours postoperatively, and three were killed later than the second postoperative day (99, A-7, and A-3 at 48, 48, and 72 hours, respectively).

Portions of the right and left hepatic lobes and one-half of each kidney were fixed in Zenker-Formol (80% Zenker's solution and 20% neutral 100% formalin) for 8 to 12 hours and washed in running water for 24 hours. All blocks were then treated in identical fashion, being dehydrated, cleared, and embedded in paraffin. Sections were cut at 5μ and stained with hematoxylin and eosin and Masson's trichrome in all instances, while in 10 cases, frozen sections of formalin-fixed tissues were stained for fat with Sudan IV.

RESULTS

Clinical.—Ten animals died either during the operation or in the immediate postoperative period, a mortality of approximately 40%. Survivors were observed to enter a phase of acute oliguria, in which the 24-hour urine volume dropped to about one-quarter of the normal as recorded in control laparotomy animals. Anuria was never encountered in these short-term experiments. Characteristic postocclusive urinary findings included albumin, trace to +; bile ±; hyaline and granular casts, 0 to 1/H. P. F.; R. B. C.'s 0 to 2/H. P. F., uncentrifuged. Preoperative urinalyses were negative in the few cases that were so investigated. The animals which survived appeared in good condition, apart from the one which died eight hours postoperatively. They were noted to take both food and drink the day of the operation and to show no untoward listlessness in excess of that shown by the laparotomy controls.

Morphological.—The macroscopic features at autopsy varied somewhat according to the procedure employed during the operation. In the group in which the porta hepatis was clamped, the livers revealed some increase in the lobular markings plus a moderate degree of pallor which imparted a mottled tawny appearance, in striking contrast to the normal reddish-brown livers of the control animals. Where ligation of either the portal vein or the hepatic artery was performed, massive, gray-green necrosis and peritonitis were found in varying stages of development, limited largely to the right lobe(s) and spreading to the left to produce focal involvement of the median lobe. In addition to induced hepatic disorders, there was found a surprisingly high incidence (43%) of naturally occurring diseases in the livers, of which coccidioidal infestation comprised more than half (27%). The kidneys showed no constant macroscopic changes, some appearing slightly hyperemic, others displaying a slight pallor. Usually they were normal in appearance.

Microscopically the hepatic lesions varied from focal through confluent to include massive necrosis, the area of involvement corresponding to the macroscopic picture. In addition, the coccidioidal foci and various lesser "natural" lesions (focal and centrolobular necrosis, serous and chronic periportal hepatitis, and cirrhosis) complicated the picture, when present.

The renal lesions correspond to those seen in human G. T. N.† As emphasized in the latter report, the lesion in acute G. T. N. is best visualized through use of the trichrome stain. The selectivity of this stain is well seen by comparison of Figures 1 and 2. In acute G.T. N. scattered glomerular capsular spaces were seen to contain proteinous fluid. There was minimal to slight swelling and desquamation of the capsular epithelium. In both the proximal and distal convoluted tubules, there were noted hyaline or colloid casts, plus a patchy, bright red, smudgy or finely granular degeneration of the cytoplasm and pyknosis of the nucleus so characteristic of acute degeneration or necrosis of the epithelium viewed in trichrome-stained sections. These epithelial changes were focal, affecting odd cells in the majority of nephrons but tending to occur in patchy groups of tubules in the

† References 1 and 2.

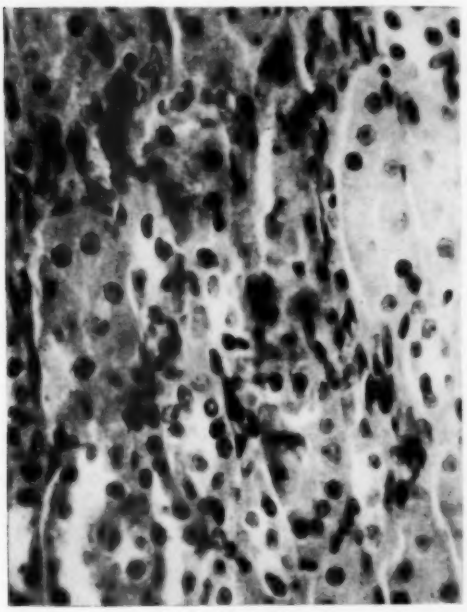
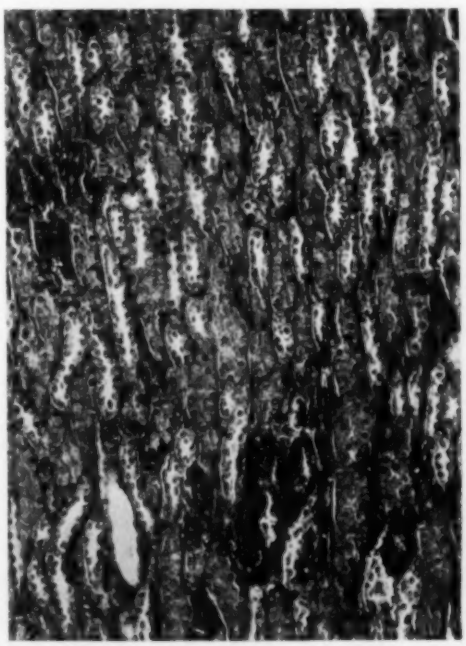


Fig. 1 (No. B-15).—Rabbit kidney. Patchy acute G. T. N. in the proximal convoluted tubules, as evidenced by nuclear pyknosis and dense eosinophilia of the cytoplasms. Note the presence of cellular cast in one distal lumen. Hematoxylin and eosin.

corticomedullary segments of the proximal tubules (Fig. 2). Red blood cell casts at various levels of the nephron were a not uncommon finding. Further features of the acute lesion are depicted in Figure 3, in addition to those seen in Figures 1 and 2. With the aid of high magnification, the cytoplasmic changes were shown to represent a disruption of the normal rod shape and polarity of the mitochondrial elements. These bodies normally stain bright red with ponceau-fuchsin, but, containing the dye within their fine fascicles, they have no profound effect on the staining intensity of the cytoplasm. When the cell is damaged, the rodlets either disrupt entirely, smearing the red-staining material throughout the cytoplasm (Fig. 4), or they fragment and agglomerate with the formation of tiny beads. These beads appear identical to those seen in mercurial renal disease, as shown by Ogilvie,[6] in 1932. The changes do not occur in postmortem autolysis. In addition to the finding of degenerate and necrotic cells "in situ," sloughed individual cells or cell clumps were easily demonstrated in the lumina at various levels of the nephron.

Some changes were interpreted as requiring more time for their development and have been referred to as subacute G. T. N. In these cases the lesions of the acute phase were often intensified. There was more pronounced swelling and sloughing of the capsular epithelium in the damaged glomeruli, and formed casts of tubular epithelium appeared in the distal and collecting tubules, amounting, in some instances, to the typical "renal failure casts" of Addis. In the later stages of the subacute process, some degree of epithelial proliferation in the glomerular tuft and Bowman's capsule; hyaline, granular, and cellular casts in distal and collecting tubules with atrophy of tubular epithelium, and early interstitial granulomatous and round-cell infiltrations were noted. Most of

Fig. 2 (No. B-15).—Rabbit kidney. Lower-power photomicrograph of same kidney from an area in close proximity to that shown in Figure 1, displaying acute G. T. N. in the corticomedullary segments of the proximal tubules. The changes show sharper contrast with surrounding normal tissue than is seen in Figure 1. Masson's trichrome stain.



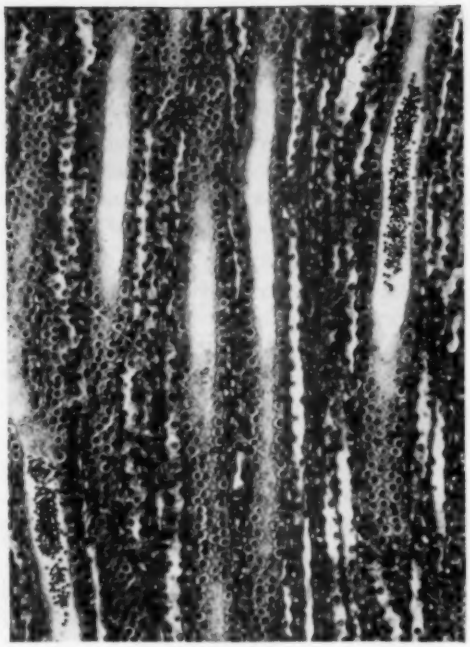


Fig. 3 (No. A-33).—Rabbit kidney. Recently formed R. B. C. casts in the lumina of collecting tubules in a case of acute G. T. N. Trichrome stain; mag. × 150.

the above features are well portrayed in Figures 5 and 6.

Correlation of Renal and Hepatic Lesions.—Owing to the high incidence of native hepatic disease and G. T. N. in the animals of the experimental group with surgical occlusion of hepatic portal structures, it was necessary to reorganize the groups for the presentation of results. Table 1 summarizes the incidence of G. T. N., correlating it with "medical" hepatic disorders in Groups A and B, and with experimental surgical hepatic lesions in Group C. Group B is composed of animals in which a surgical procedure accompanied the medical lesion in the liver. Group D consists of unoperated controls, while Group E contains both simple laparotomy controls and animals which underwent surgery but died under the anesthetic. Group F consists of two animals in which temporary (10-minute) occlusion of the left renal pedicle was performed during the course of a 20-minute laparotomy. Table 2 presents the detailed findings on individual rabbits. It records the surgical procedure, if any; the presence or absence of complicat-

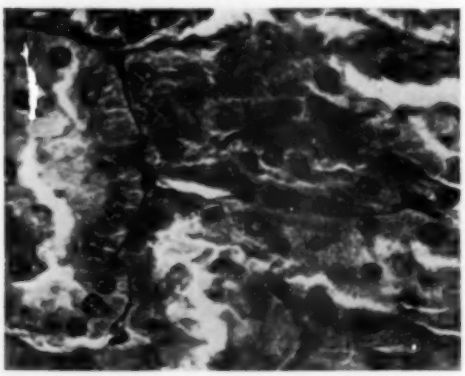


Fig. 4 (No. A-8).—Rabbit kidney. Acute G. T. N. The group of three contiguous pyknotic nuclei mark acutely degenerated cells in which the mitochondria have disappeared and their substance is smeared throughout the cytoplasm. Trichrome stain; reduced 3/10 from mag. × 750.

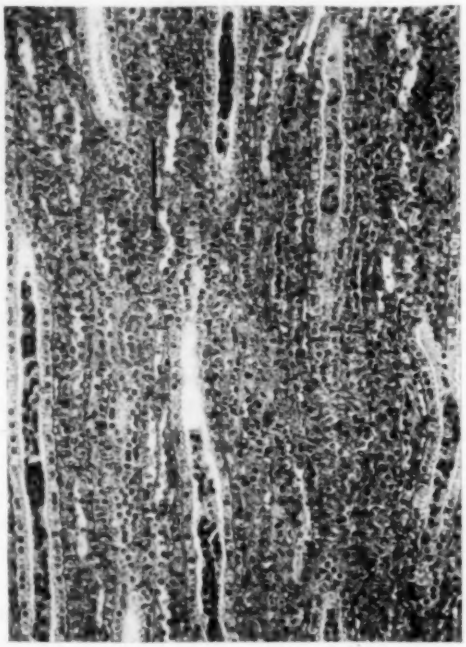


Fig. 5 (No. A-41).—Rabbit kidney. Subacute G. T. N. Large mixed cellular and granular casts are seen plugging the collecting tubules. That they are of fairly recent origin is borne out by the good state of preservation of the component cells. Trichrome stain; mag. × 150.

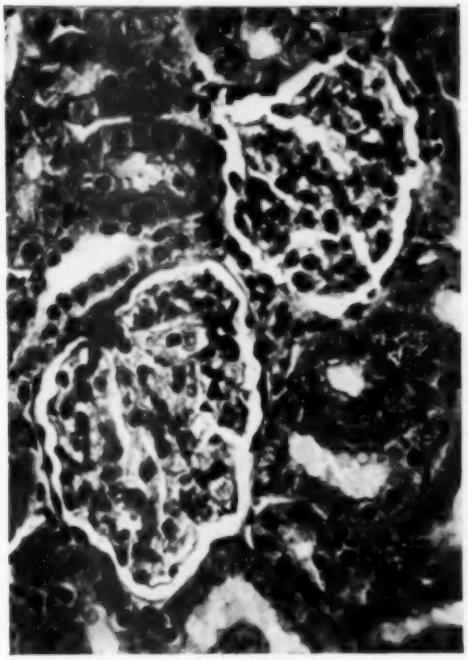


Fig. 6 (No. B-24).—Rabbit kidney. Subacute G. T. N. Proliferation of the epithelium of the tuft and capsule, with synechiae, is seen in the smaller glomerulus. Several tubules contain proteinous fluid. Trichrome stain; mag. × 400.

ing hepatic disease; the weight of the animal; its clinical course; the histopathology of the liver; the postoperative urinalysis, where applicable, and the degree of G. T. N. In the recording of the latter, — indicates the absence of any form of G. T. N.; + and ++ indicate the acute phase of G. T. N. graded according to numerical distribution among the nephrons, and +++ and ++++ indicate subacute G. T. N. graded in like manner.

Of the 31 animals with liver damage listed in Groups A, B, and C, Table 1, 29 displayed varying degrees of G. T. N. Of the two which failed to show renal damage, one revealed slight coccidioidosis of the liver and the other had undergone clamping of the porta hepatis for a 10-minute period with no resultant liver damage. Postoperative urinalysis revealed renal damage in 12 of the 13 cases so investigated. Their preoperative urine specimens were normal. In the purely surgical group (C), the hepatic lesion was invariably some form of necrosis. Though usually focal and patchy following clamping of the porta hepatis, several exceptions with severer lesions such as centrolobular, confluent, and massive necrosis were present. Where ligation of one or another of the vascular components of the porta was performed, massive necrosis was the inevitable sequela.

Seventeen of the 18 animals in Groups D, E, and F had no lesions of the liver or kidneys apart from the unilateral acute G. T. N. occurring in the two cases in Group F after clamping off the left renal pedicle for a 10-minute period. A remaining animal in Group D did show the lesions of acute G. T. N. in the absence of provable hepatic damage. The liver in this instance contained tiny foci of acute degeneration, marked by nuclear pyknosis and cytoplasmic polychromasia, but necrosis was lacking.

Thus it was found that G. T. N. correlated with disease of the liver in roughly 94% of experimental animals, of which approximately 63% of the liver disease was of nonsurgical etiology. In this study, it occurred in otherwise normal rabbit kidneys without proved hepatic disease in approximately 2% of cases.

In order to establish the identity of the histopathology of acute G. T. N. with that seen in true renal ischemia, the left renal pedicles were clamped off in the two rabbits of Group F for 10-minute periods. The

TABLE 1.—*Summary of the Incidence and Correlation of Hepatic and Renal Lesions*

	No.	No. with G.T.N.
Rabbits with Liver Damage		
Group A—Medical diseases of liver.....	8	7
Group B—Medical and surgical diseases of liver	13	13
Group C—Surgical diseases of liver....	10	9
Total	31	29
Rabbits Without Liver Damage		
Group D—Unoperated controls	9	1
Group E—Laparotomy controls and operative deaths	7	0
Group F—Laparotomy and occlusion of left renal artery.....	2	0, R. kidney 2, L. kidney
Total	18	1

TABLE 2.—*Incidence of Experimental and Natural Glomerulotubular Nephrosis in Rabbits*

Group	Rabbit No.	Surgical Procedure	Medical Disease of Liver	Wt., in Gm.	Clinical Course	Hepatic Lesion	Post-operative Urinalysis	Degree of G.T.N.*
Medical diseases of liver (8)								
A	A22	Nil	Coccidioidosis	1860	K & A †	Coccidioidal mycosis and focal necrosis	—	+
	A23	Nil	Coccidioidosis	2030	K & A	Coccidioidal mycosis and focal necrosis and portal fibrosis	—	+++
	B13	Nil	Coccidioidosis	2300	K & A	Coccidioidal mycosis (extreme)	—	+
	B15	Nil	? Infectious hepatitis	1320	K & A	Serous hepatitis and centrolobular necrosis	—	+
	B16	Nil	? Infectious hepatitis	1350	K & A	Centrolobular necrosis	—	++
	B19	Nil	Coccidioidosis	2200	K & A	Coccidioidal mycosis (slight)	—	—
	B23	Nil	? Infectious hepatitis	2300	K & A	Focal necrosis (marked)	—	++
	B24	Nil	Coccidioidosis	2170	K & A	Coccidioidal mycosis (extreme)	—	++++
Medical and surgical diseases of liver (13)								
B	98	Porta clamped 30 min.	? Subacute hepatitis	1920	Operative death	Periportal fibrosis (early)	—	+
	A1	Porta clamped 23 min.	? Infectious hepatitis	2010	Operative death	Focal necrosis (patchy)	—	+++
	A4	Portal vein ligated	Coccidioidosis	2020	Early post-operative death	Focal necrosis (massive) and coccidioidosis	—	+++
	A6	Hepatic artery ligated	Coccidioidosis	2050	Death 8 hr. postop.	Centrolobular necrosis and coccidioidal mycosis	—	+
	A27	Laparotomy for 20 min.	? Acute and subacute hepatitis	1950	K & A 24 hr. postop.	Focal necrosis (patchy) and periportal fibrosis	Neg.	+
	A28	Laparotomy for 20 min.	Coccidioidosis	2100	Operative death	Focal necrosis (patchy) and coccidioidal mycosis	—	++
	A38	Porta clamped 15 min.	Coccidioidosis	1730	Operative death	Focal necrosis (patchy) and coccidioidal mycosis	—	+
	A39	Porta clamped 18 min.	Coccidioidosis	1350	K & A 24 hr. postop.	Focal necrosis (massive) and coccidioidal mycosis	Alb. + Bile tr. R.B.C. 2/HPF Casts 0-1/HPF	+
	A41	Porta clamped 14 min.	? Acute and subacute hepatitis	1980	Operative death	Focal necrosis (patchy) and periportal fibrosis	—	++++
	A42	Porta clamped 20 min.	? Infectious hepatitis	1860	Operative death	Centrolobular necrosis	—	+
	A43	Porta clamped 20 min.	? Infectious hepatitis	1730	Operative death	Centrolobular necrosis	—	+
	A35	Porta clamped 20 min.	Coccidioidosis	2480	K & A 24 hr. postop.	Focal necrosis (patchy) and coccidioidal mycosis	Alb. + Bile — R.B.C. 0-2/HPF Casts 0-1/HPF	++
	A36	Porta clamped 15 min.	Coccidioidosis	1760	K & A 24 hr. postop.	Focal necrosis (patchy) and coccidioidal mycosis	Alb. + Bile — R.B.C. 0-1/HPF Casts 0-1/HPF	+
Surgical diseases of the liver (10)								
C	99	Porta clamped 30 min.	Nil	1790	K & A 48 hr. postop.	Focal necrosis (patchy)	Alb. tr. Bile + R.B.C. — Casts —	++
	A3	Portal vein ligated	Nil	1850	K & A 72 hr. postop.	Necrosis: R. lobe, massive; M. lobe, focal	Alb. — Bile + R.B.C. 2/HPF Casts 0/HPF	++
	A5	Porta clamped 10 min.	Nil	1530	K & A 24 hr. postop.	Centrolobular necrosis (hemorrhagic)	Alb. + Bile + R.B.C. 0-1/HPF Casts 0-1/HPF	++

TABLE 2.—*Incidence of Experimental and Natural Glomerulotubular Nephrosis in Rabbits*—Continued

Group	Rabbit No.	Surgical Procedure	Medical Disease of Liver	Wt., in Gm.	Clinical Course	Hepatic Lesion	Post-operative Urinalysis	Degree of G.T.N.*
	A7	(Rt.) Hepatic artery ligated	Nil	2050	K & A 48 hr. postop.	Necrosis: R. lobe, massive; M. lobe, focal	Alb. + Bile + R.B.C. 0-2/HPF Casts 0-1/HPF	++
	A8	Porta clamped 20 min.	Nil	2150	K & A 30 hr. postop.	Focal necrosis (massive)	Alb. + Bile — R.B.C. 0-1/HPF Casts 0-1/HPF	++
	A32	Porta clamped 20 min.	Nil	1920	K & A 24 hr. postop.	Focal necrosis (patchy)	Alb. tr. Bile tr. R.B.C. 0-1/HPF Casts 0-1/HPF	+
	A33	Porta clamped 20 min.	Nil	2540	K & A 24 hr. postop.	Focal necrosis (patchy)	Alb. + Bile tr. R.B.C. 0-2/HPF Casts 0-2/HPF	++
	A34	Porta clamped 10 min.	Nil	1830	K & A 24 hr. postop.	Normal	Neg.	—
	A37	Porta clamped 20 min.	Nil	2100	K & A 24 hr. postop.	Focal necrosis (patchy)	Alb. tr. Bile tr. R.B.C. 0-1/HPF Casts 0-1/HPF	+
	A40	Porta clamped 20 min.	Nil	2050	K & A 24 hr. postop.	Focal necrosis (massive)	Alb. + Bile tr. R.B.C. 0-1/HPF Casts 0/HPF	+
Untreated controls (9)								
D	A20	Nil	Nil	2050	K & A	Nil	—	—
	A21	Nil	Nil	1930	K & A	Slight chronic peri-portal hepatitis	—	—
	A24	Nil	Nil	1700	K & A	Nil	—	—
	A25	Nil	Nil	1900	K & A	Nil	—	—
	B14	Nil	Nil	2400	K & A	Nil	—	—
	B17	Nil	Nil	2200	K & A	Nil	—	—
	B18	Nil	Nil	2500	K & A	Nil	—	—
	B20	Nil	Nil	2500	K & A	Tiny foci of ? acute degeneration	—	+
	B21	Nil	Nil	2450	K & A	Nil	—	—
Operative deaths and laparotomy controls (7)								
E	100	Porta hepatis ligated	Nil	1850	Operative death	Nil	—	—
	A2	Porta clamped 6 min.	Nil	1730	Operative death	Nil	—	—
	A26	Laparotomy for 20 min.	Nil	2580	K & A 24 hr. postop.	Nil	Neg.	—
	A29	Laparotomy for 20 min.	Nil	2440	K & A 24 hr. postop.	Nil	Neg.	—
	A44	Laparotomy for 20 min.	Nil	2260	K & A 24 hr. postop.	Nil	Neg.	—
	A45	Laparotomy for 20 min.	Nil	2400	K & A 24 hr. postop.	Nil	Neg.	—
	A46	Laparotomy for 20 min.	Nil	2210	K & A 24 hr. postop.	Nil	Neg.	—
Laparotomy and occlusion of left renal artery (2)								
F	A30	Laparotomy for 20 min. Clamp L. renal pedicle for 10 min.	Nil	2530	K & A 24 hr. postop.	Nil	Neg.	R — L ++
	A31	Laparotomy for 20 min. Clamp L. renal pedicle for 10 min.	Nil	1880	K & A 24 hr. postop.	Nil	Alb. tr. Bile — R.B.C. 0-2/HPF	R — L ++

* Glomerulotubular nephrosis. Degree: Absent —; Minimal acute GTN +; Moderate acute GTN ++; Moderate subacute GTN +++; Advanced subacute GTN ++++.
† Killed and autopsied.

animals were otherwise treated as simple laparotomy controls, as outlined previously. Only one animal developed abnormal urinary constituents, but both showed a well-developed focal renal lesion, indistinguishable from acute G. T. N., in sections from the left kidneys (Fig. 7). The right kidneys were normal.

Variable results were observed in the 10 cases stained for fat, in both the kidney and the liver. It would appear that fatty degeneration is not a feature of the acute or subacute lesion of G. T. N. and that the fat

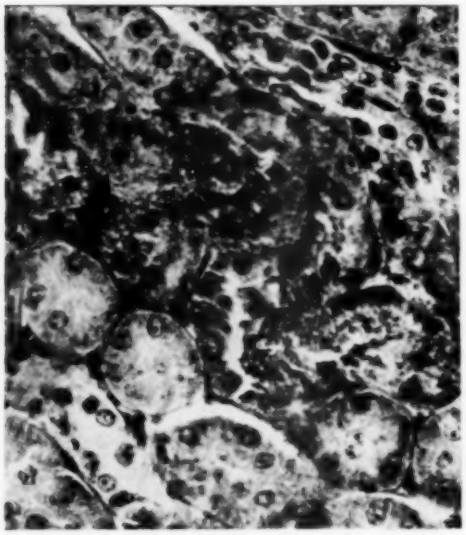


Fig. 7 (No. A-31).—Rabbit kidney. Anoxic nephrosis. The tubular lesion is identical with that of acute G. T. N. as shown in Figures 1, 2, and 4. Trichrome stain; mag. × 400.

content of the liver is independent of all of the disease processes encountered in this series.

COMMENT

This investigation establishes an impressive correlation between the presence of liver damage and a renal lesion designated as glomerulotubular nephrosis. A similar correlation in human autopsy material was noted in Part II [2] of this series. Because of this latter observation, the present experiments were first designed to study the effect of experimental acute uncomplicated liver damage on structure and function of the kidney. However, the high incidence of natural disease in the liver made it impossible to analyze the effects of uncomplicated acute liver damage on the kidneys, and on final evaluation of this experiment it was clear that natural disease of the liver, alone or in combination with simple laparotomy or terminal acute surgical hepatic damage, as well as acute surgical damage of the liver alone, was correlated with renal G. T. N. This was in contrast to the absence of renal G. T. N. lesions in animals with no natural or experimental liver disease. This unexpected finding in the rabbit of the correlation of natural disease of the liver and G. T. N. lesions increases the significance of the association of liver damage and renal G. T. N.

Extreme liver dysfunction with acute renal failure has been recognized for some time under the term hepatorenal syndrome. Renal lesions of this entity possess the basic features seen in so-called "lower nephron nephrosis." In this and in the preceding paper, in detailing the morphology of G. T. N. lesions it has been pointed out that the elementary changes are qualitatively identical to those described by Oliver [7] in dissected nephrons from cases of acute renal failure. Many investigators have attempted to duplicate experimentally the clinical and pathological features of the hepatorenal syndrome.‡ In most of their experiments the liver lesions were usually chronic and have been complicated by cholemia and shock. The present study was planned to investigate the effect of uncomplicated liver damage on the kidney without producing such profound changes as seen in the hepatorenal syndrome. In this way it was hoped to isolate more exactly the role of the liver in damage to the kidney than could be determined from natural human disease or experiments where more profound metabolic changes such as shock and cholemia are occurring. The findings of the present experiments suggest that minimal disease and dysfunction of the liver produce a microcosm of the hepatorenal

‡ References 3, 4, and 5.

syndrome and indicate that liver damage may be an essential factor in the genesis of the G. T. N. lesion, which is basically similar to the renal lesion of the hepatorenal syndrome. These findings may indicate that liver function influences kidney function and structure in some unexplained way.

In the present study, it would be interesting to know the ultimate mechanisms responsible for the renal lesion and the relationship of liver damage to these mechanisms. That the renal lesions could be due to renal ischemia seems reasonable. They have the essential elements of "lower nephron nephrosis," in which there is good evidence for ischemic pathogenesis, as noted by Tomb,[8] Oliver and co-workers,[7] Sheehan and Moore,[9] and Bull and Dible.[10] Furthermore, in two of our rabbits temporary clamping of the renal pedicle produced a similar lesion. On the other hand, in the present study, while it seems reasonable that renal vasospasm might accompany acute surgical damage of the liver, credulity is strained to suppose renal vasospasm accompanies chronic natural disease of the liver. In any case, to prove that the lesion of glomerulotubular nephrosis is of ischemic genesis and a forerunner of "lower nephron nephrosis," it would be necessary to make serial time studies on the development of experimental "lower nephron nephrosis" with its accompanying renal vasospasm and determine if the early lesions were identical to G. T. N. A parallel line of investigation is at present being conducted by one of us (C. N. C.) which involves acute carbon tetrachloride intoxication in the rat. A renal lesion identical to acute G. T. N. occurs in the latter experiments. Lesions which vary in intensity from albuminuria and casts in the collecting tubules, through acute tubular necrosis to focal and generalized renal cortical necrosis, have been reported by Sheehan and Moore [9] in eclampsia and uteroplacental apoplexy in humans, and by Block and coworkers § in exsanguination studies in the dog and rat. The mild and intermediate forms of these lesions would appear to be identical to acute G. T. N. In both instances the authors attribute the etiology of the renal lesions to vasospasm.

Whatever the ultimate mechanism of the G. T. N. lesion, it is important to assess the role of the liver in the over-all chain of events. In severe liver disease with the development of the hepatorenal syndrome and in severe experimental liver damage, it has been suggested by Furtwaengler (cited in Pytel [5]) that renal vasospasm is mediated through hepatic dysfunction by the accumulation of a nephrotoxin which acts on the walls of blood vessels. In Part II [2] of this series we have likewise stressed the plausibility of such a pathogenetic mechanism. However, in the present investigation there is no proof that the damage to the liver results in abnormal vasospastic materials in the circulation, nor can temporary renal vasospasm secondary to acute temporary shock accompanying the surgical damage of the liver be ruled out, though the occurrence of G. T. N. without shock in natural liver disease tends to minimize the importance of the latter.

To establish that the genesis of the renal damage is secondary to liver damage by way of either the accumulation of renotoxic or renal vasopressor substances requires further experimentation. The salient facts which have emerged from these studies are the close correlations between renal G. T. N. and hepatic damage, whether natural, experimental, or both. Even more unusual is the fact that the natural diseases were, on the whole, minimal in degree and could not be expected to produce severe, generalized, nonspecific disturbances of homeostasis. Such a finding suggests that there is something unique about damage to the liver as contrasted with damage to other tissues.

Throughout the present and the earlier investigations we have stressed the important contribution of the Masson trichrome stain. Through this medium, renal tissue which may at first sight appear normal in hematoxylin and eosin sections will often reveal acute damage, changes which can subse-

§ References 11 and 12.

quently be traced out in hematoxylin and eosin sections. The stain requires considerable technical skill, the chief source of error lying in the differentiation with picric alcohol. This step is not complete until all hematoxylin has been removed from the cytoplasm of the epithelial cells of the tubules, as its presence in this site tends to mask the subsequent uptake of red dye from the ponceau-fuchsin stain.|| The timing of this step is aided by the simultaneous disappearance of hematoxylin from the cytoplasm of glomerular tufts and its practical disappearance from tubular epithelial nuclei.

SUMMARY

An attempt has been made to reproduce experimentally correlated hepatic and renal damage corresponding to the associated hepatic and renal lesions reported in human autopsy material in Part II of this series. This was done by means of temporary occlusion of the hepatic vasculature in the rabbit. The object of this procedure was to produce a state of acute hepatic insufficiency without jaundice. Glomerulotubular nephrosis was found to develop in 90% of these animals.

A rather large number of the rabbits used in this investigation (43%) showed preexisting hepatic lesions of varying types and duration. These hepatic changes showed a 95% correlation with degenerative renal changes, causing major reorganization of the experimental groupings.

A lesion identical to acute glomerulotubular nephrosis has been produced in two rabbits by direct renal ischemia.

The proposed ischemic etiology of G. T. N. has been discussed in the light of the associated hepatic disorders, a possible sequence of events being as follows: Hepatic insufficiency develops in the presence of a variety of lesions; the insufficient liver fails to detoxify some potent vasospastic material in the circulation; this agent produces selective arteriospasm in small renal vessels.

The Masson trichrome stain is recommended for the investigation of acute degenerative lesions of the kidney, and a note is included on some of its more important technical features.

The experimental aspects of this investigation were performed at Queen's University. All photographs were taken by Mr. T. C. Dodds, Department of Pathology, Photomicrography Unit, University of Edinburgh.

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|| We have found the most satisfactory ponceau dye to be "Ponceau 2 R," color-index 79, by Harleco.

Intramucosal Carcinoma of the Esophagus

Report of a Case

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The concept of carcinoma in situ has received a great deal of attention in current medical literature * and has been extended in scope to many organs, including uterus, stomach, lung, and skin. Carcinoma of the esophagus is a relatively common neoplasm, but comparatively few studies have been reported on intramucosal tumors. Imbriglia and Lopusniak [5] reported a case of carcinoma in situ of the esophagus, with ulceration but without invasion. Burgess and co-workers [3] made a special study of 88 carcinomas of the esophagus and found intramucosal spread beyond evidence of gross tumor ranging from 1 to 4 cm. in 15 cases. Several reports by Stout † suggest an increasing frequency of the superficial spreading type of carcinoma of the stomach (23 cases between 1937 and 1945 at Presbyterian Hospital). Other authors have described multiple small carcinomas arising in a bed of altered mucosa ‡ or have mentioned briefly the mucosal spread of cancer.§ The case we are reporting is remarkable in that it showed involvement of almost the entire esophageal mucosa without a localized mass and with only limited superficial invasion of the submucosa and muscularis. No symptoms referable to the esophagus were recognized during life.

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From the Pepper Laboratory of Clinical Medicine, the Laboratory of Surgical Pathology, and the Penn Mutual Life Insurance Company Foundation for the Study of Neoplastic Disease, Hospital of the University of Pennsylvania.

* References 1, 4, and 6.

† References 10, 11, and 12.

‡ References 9 and 14.

§ References 2 and 8.

REPORT OF CASE

The patient, a 53-year-old presser, was admitted to the Hospital of the University of Pennsylvania on June 3, 1953, with a lump on the right side of his neck, just below the mandible, which had been present for about two years but which had suddenly increased in size. He had no particular complaints but admitted to occasional burning or soreness in the epigastrium which radiated around the left costal margin and occasionally to the back. This was somewhat relieved by food. Physical examination was negative except for a mass (12×8×5 cm.) in the angle of the right mastoid-mandibular region. The mass was firm, fixed, and nonfluctuant. Biopsy proved it to be a well-differentiated squamous cell epithelioma with extensive keratinization. The primary source of the tumor was undetermined, although a careful search was made of the ears, nasal and oral pharynx, and larynx. On roentgen examination the swallowing function was normal, as was the chest examination. In the belief that the primary tumor was probably of parotid salivary gland origin, radical neck dissection was carried out in combination with resection of the parotid (Fig. 1). Twenty-one of the 24 lymph nodes identified in the specimen contained metastatic tumor identical with that involving the parotid salivary gland. It was impossible, however, to determine whether or not the tumor was a primary salivary gland tumor. The patient was discharged June 25, to return for x-ray therapy, and in August and September received a total of 4000 r in air to the right supraclavicular lymph nodes and right parotid area.

The patient was admitted for the third time on Sept. 27, with profuse bleeding from a fistula which had developed between the skin and pharynx following the x-ray therapy. He was in a state of shock on arrival. The right carotid artery was ligated, and four days later a gastrostomy was performed because the fistula interfered with swallowing. He was discharged again on Oct. 1.

The patient was readmitted a month later because of respiratory difficulty of increasing severity. By this time he was markedly emaciated, and there was sloughing of the skin over a large area over the right side of the neck and mandible. Numerous hard, discrete nodes (2 to 4 cm.) were palpable in the left side of the neck, in both axillae, and in the supraclavicular fossae. He was unable to talk because of trismus. A tracheotomy was

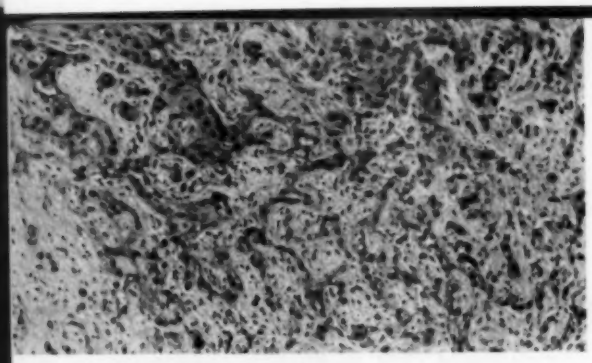


Fig. 1.—Surgical specimen showing well-differentiated epithelioma; × 150.

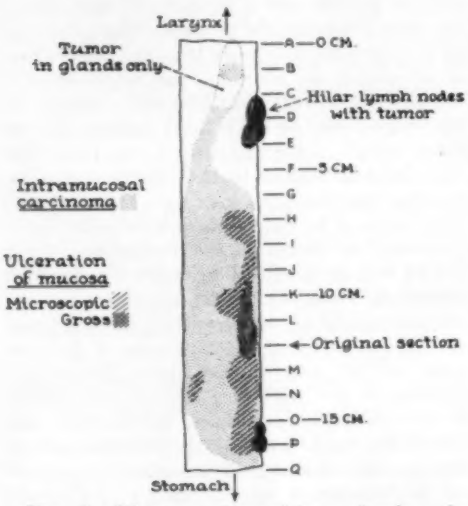


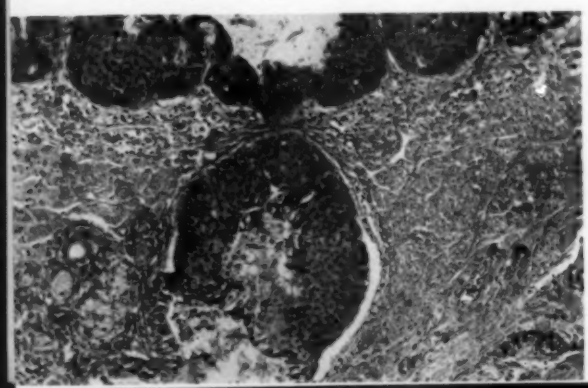
Fig. 2.—Diagram of esophagus showing the spread of carcinoma.

done on Nov. 3. Repeated aspirations plus antibiotic therapy had some beneficial effects, but the patient gradually grew weaker and died quietly on Nov. 30, two years after the onset of his illness and nine months after his first admission to the Hospital of the University of Pennsylvania.

NECROPSY

Postmortem examination showed an emaciated Negro man, 166 cm. tall, weighing 40 kg. On the right side of the neck there was a 10 cm. ulcer exposing the mandible and carotid vessels. Numerous firm lymph nodes were palpable in both axillae and both sides of the neck, including a 6 cm. mass at the angle of the left mandible. A gastrostomy tube pierced the abdominal wall and a tracheotomy opening was present in the neck. Examination of the thoracic cavity revealed metastatic tumor nodules measuring up to 2 cm. in diameter, involving the visceral pleura and invading the lung parenchyma superficially. In addition, the tracheal and hilar lymph nodes and a small group of nodes adjacent to the lower esophagus were greatly enlarged by metastatic tumor. The trachea and bronchi were not involved. Embedded in the muscle of the apex of the right ventricle of the heart was a tumor nodule 2 cm. in diameter. The esophagus showed no gross evidence of tumor, although a superficial area of ulceration 5 cm. from the distal end was noted. This area was slightly thickened, and a section through it was taken because of the ulceration. The stomach showed only the gastrostomy opening in the anterior wall, and the rest of the abdominal organs, including the liver, were not remarkable. No enlarged lymph nodes were observed in the abdominal cavity.

Fig. 3.—Carcinoma extending into esophageal glands (Level B); × 150.



Microscopic examination confirmed the presence of squamous cell carcinoma in the pleural nodules, hilar and cervical nodes, and the myocardium. The esophagus showed intramucosal carcinoma adjacent to the ulcer, and this led to more detailed examination of the entire esophagus. The fixed esophagus, which measured 17 by 3 cm., was sectioned at 1 cm. intervals (18 sections). A reconstruction then was made of the extent of the tumor (Fig. 2). The carcinoma was found to involve the mucosa of most of the esophagus with extension into the esophageal glands in many places. No invasion of the muscularis was found except for a single small area at Level L. Superficial invasion of the submucosa was found at Levels I through L.

At the upper end of the esophagus near the larynx (Level A) the epithelium showed some hyperchromatism and loss of nuclear polarity in the basal layers, with infiltration

of squamous epithelial cells beneath the columnar epithelium of the esophageal glands. One centimeter lower (Level B) approximately one-third of the circumference of the esophagus showed intramucosal carcinoma with extension into the glands (Fig. 3). At Level C only an occasional submucosal gland was involved, while at levels D, E, and F a 5 mm. strip of intramucosal carcinoma was found (Fig. 4). At levels G, H, I, and J, virtually the entire mucosa of the esophagus had undergone neoplastic transformation, with only superficial invasion of the submucosa at Levels I and J. The esophageal glands were cystically dilated, and some were invaded by tumor cells. At levels K and L most of the surface epithelium was eroded but the submucosal glands were filled with carcinoma cells. In addition, Level L showed invasion of the muscularis (Fig. 5). This was the level of the superficial ulceration seen grossly. At levels M and N patches of normal mucosa were seen adjacent to cancerous areas, but most of the mucosa was lost. Sections O and P (Fig. 6) showed progressively less involvement of the surface epithelium until, in the section closest to the stomach (Q), only a tiny area of surface epithelium and a rare submucosal gland were involved.

Lymph nodes from the hilar area of the lung adjacent to Level D were virtually replaced by malignant squamous cells forming numerous keratin pearls. Lymph nodes adjacent to the lower esophagus (Level P) contained a few tumor elements but showed chiefly plasmacytic infiltration. Chronic inflammatory changes were present in the submucosa of the ulcerated areas and chronic inflammatory exudate in the perineural lymphatics at higher levels. An occasional group of tumor cells was found in the serosal lymphatics.

COMMENT

The extensive but superficial character of the mucosal involvement aroused speculation about the origin and spread of this tumor. In view of the widespread character of the lesion and the relative lack of invasiveness, it seems unlikely that it began with malignant

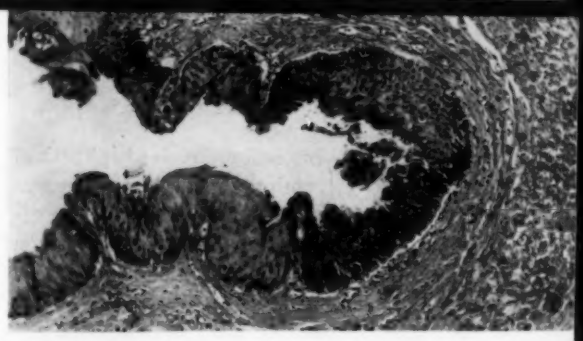


Fig. 4.—Normal esophageal mucosa is seen at the top with transition to intramucosal carcinoma below and in center (Level O); × 150.

change in a single cell or group of cells and spread by multiplication of these cells in centrifugal fashion. It seems more likely that it arose either as part of a general carcinomatous change in the mucosa of the esophagus in response to a still unknown stimulus ‖ or as multiple areas of intramucosal carcinoma, which subsequently spread along the mucosal surface to form a confluent superficial lesion. The epithelium of the esophageal glands was undermined by infiltration of malignant squamous cells (Fig. 3), but the squamous epithelium did not show similar

‖ References 15 and 16.

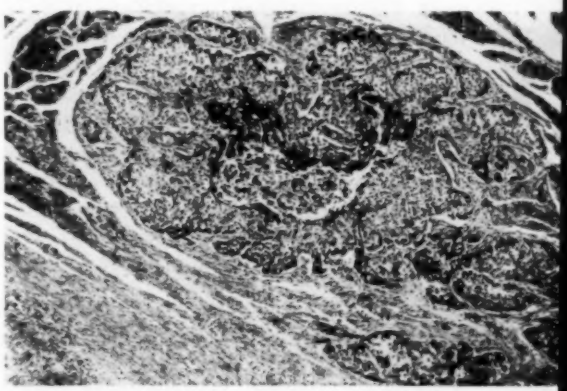


Fig. 5.—Invasion of muscularis and submucosa is at lower left (Level L); × 75.

Fig. 6.—Transition zone between normal esophageal mucosa and carcinoma (Level O); × 150.

undermining. Instead, uninvolved epithelium was in direct continuity with the carcinomatous areas. Although the transition appears abrupt in places, the change was much more gradual in the basal cell layer than in the more superficial layers. In fact, the basal cell layer of most of the esophagus showed some alteration, such as close packing of cells with rounding-up of nuclei and clarity of surrounding cytoplasm. The evidence, then, suggests a widespread carcinomatous transformation of the esophageal mucosa with extension into the esophageal glands.

The striking absence of symptoms usually associated with esophageal carcinoma is easily understood. At necropsy, the esophagus appeared grossly normal, except for discoloration of the mucosa of the distal third and a small area of superficial ulceration much like that not infrequently seen after long illnesses. Although symptoms of esophageal involvement can be noted in retrospect, these were obscured during life by the prominence of the disease in the neck and, subsequently, by postirradiation complications. The aggravation of symptoms by solid foods and relief from alkaline fluids suggest an esophageal lesion. In only 4 of the 124 cases of esophageal carcinoma reviewed by Taquino [13] was the diagnosis missed during life, and in these the principal symptoms arose from massive liver, cerebral, and lung metastasis, and massive hemorrhage from erosion into the aorta, respectively. The average duration of symptoms was between 4.2 and 5.5 months, depending upon the location of the primary lesion. Picard and coworkers [7] have described a case of carcinomatous infiltration of almost the entire esophagus without symptoms of dysphagia.

SUMMARY

A case of carcinoma of the esophagus is presented showing intramucosal involvement of almost the entire esophagus without dysphagia. The presenting symptoms were related to cervical lymph node metastasis.

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Rupture of the Pulmonary Artery Complicating Rheumatic Mitral Stenosis

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Although several cases of rupture of the pulmonary artery have been reported, a review of the literature failed to reveal an instance in which mitral stenosis was the underlying cause. In the following report a case of advanced mitral stenosis is presented in which death was due to rupture of the main pulmonary artery.

REPORT OF CASE

Clinical Summary.—A 42-year-old married white woman was first seen six months before her death, complaining of anterior chest pain and breathlessness.

At the age of 14 she had had a rheumatic infection characterized by swollen joints and fever, on account of which she was confined to bed for several weeks. There were no recurrences. She married and had seven pregnancies; of the offspring five were full-term healthy children and two were prematures who died in infancy.

About two years before her death she began to note shortness of breath, which increased in degree until she was dyspneic while walking on the level. She could, nevertheless, lie flat in bed without discomfort. Coincident with this symptom she became increasingly subject to fatigue and began to have edema of her legs upon standing.

About one year before her death she first noted chest pain on exertion. This was felt in the midsternal area and radiated to the left arm. It came only with fairly vigorous exercise and lasted only five minutes after she began to rest. It was dull and aching or constrictive in character.

Examination showed her to be thin, chronically ill, dyspneic on slight exertion, moderately cyanotic, and moderately jaundiced. She had an active recurrent iritis and keratitis. A few rales were audible at both bases. There was a moderate kyphosis without fixation. The pulse was regular at rate 96 and was equal at the two wrists. The blood pressure was 120/80. The heart was grossly enlarged. No thrills were palpable. The pulmonic second sound exceeded the aortic second, and the first mitral sound was accentuated. There was a low-pitched Grade 2 pulmonic systolic murmur, and there was a high-pitched Grade 3 diastolic murmur heard in the pulmonic area and for a short distance down the left border of the sternum. A Grade 2 systolic murmur was audible at the apex and in the axilla, and there was a low-pitched rumbling middiastolic murmur with presystolic accentuation confined to the apical region. The liver edge was palpable 6 cm. below the right costal margin. Edema was absent. Atrophy of the right deltoid and right peroneal group of muscles indicated old poliomyelitis.

An electrocardiogram showed right ventricular hypertrophy. Fluoroscopic examination revealed a large heart, with a marked prominence of the pulmonary artery and dilatation of the branches. The pulsation in these arteries was vigorous but not extreme. The auricles were large, the left apparently no larger than the right. The clinical findings were thought to be consistent with an interauricular septal defect with superimposed mitral stenosis of rheumatic origin (the Lutembacher syndrome).

The patient was treated with a low-salt diet and digitalis. She felt fairly well for a few months but then, in spite of continued treatment, she became dyspneic at rest and edematous, requiring hospitalization. On the 10th day after admission she suddenly began to gasp, lapsed into coma, and died within 10 minutes.

Laboratory Examinations.—Hemoglobin, 20.2 gm.; red cell count, 5,200,000 per cubic millimeter.

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Fig. 1.—Dilated pulmonary artery with a fresh adventitial hemorrhage and small punctate perforation.

The blood film showed macrocytosis, polychromatophilia, and a few nucleated red blood cells. White cell count, 13,200 per cubic millimeter, with normal differential. Serum bilirubin, 8.6 mg., 6.2 mg., and 4.0 mg. per 100 cc. at three-day intervals, but the remainder of the liver function tests were normal. Bile was present in the urine.

Autopsy Findings (performed 14 hours after death).—The subject was a thin adult white woman, showing a mild degree of jaundice. There were 300 ml. of clear amber fluid in the right pleural cavity and 200 ml. on the left side. The peritoneal cavity contained about 500 ml. of similar fluid.

The pericardial sac was filled with liquid blood and several large fresh clots. The pulmonary artery was markedly dilated, and an anterior perforation was seen 1.5 cm. above the heart (Fig. 1) from which blood oozed into the pericardial sac. As shown in the photograph, a fresh adventitial hemorrhage was present above the perforation. The pulmonary artery measured 14.0 cm. at its greatest circumference. The ascending aorta measured 7.0 cm. in circumference. When the pulmonary artery was opened posteriorly, an extensive irregular tear was seen on the intimal surface at the point of rupture (Fig. 2). The longer horizontal tear measured 5.0 cm. in length. The tear extended through the wall to the adventitia, which showed the smaller vertical tear seen in Figure 1. Just above the pulmonary valve an oblique irregular tear measuring 1.5 cm. in length was also seen, which mainly involved the intima. Elsewhere the intima was very rough and granular. A rather wide depressed scar can also be seen in Figure 2, which had the appearance of a previous healed tear. The major branches of the pulmonary artery were also dilated, and throughout the pulmonary arterial tree numerous atheromatous plaques were seen.

The heart weighed 360 gm. and showed marked right ventricular dilatation and hypertrophy. The average width of the right ventricular wall was 1.0 cm., and the papillary muscles were considerably larger than those seen in the left ventricle. The left ventricle was neither hypertrophied nor dilated. The mitral valve showed a marked funnel-shaped stenosis, which would not admit the tip of the small finger. There was marked thickening and adhesion of the chordae tendineae. Both the aortic and the pulmonary valve ring measured 7.0 cm. in circumference, and the cusps were normal. The tricuspid valve ring measured 12 cm. Some encroachment upon the pulmonary infundibulum was caused by the enlarged papillary muscles of the right ventricle. No congenital abnormalities of the heart or great vessels were present. The coronary vessels and the aorta were free of atherosclerosis.

The right lung weighed 690 gm. and the left 480 gm. In the periphery of the right lower lobe a firm red infarct was found, which had a 6.0 cm. base on the inferior surface. In the branch of the pulmonary artery to this area, a loose antemortem embolus measuring 0.5 cm. in diameter was found. The lung parenchyma was dry, and the only other abnormality seen was prominence of the pulmonary arterial branches which pouted on the cut surface.

The liver weighed 1000 gm. and showed a characteristic picture of advanced chronic venous congestion with mild cardiac cirrhosis. The remainder of the abdominal organs showed only the changes of chronic venous congestion.

Microscopic Examination.—Sections from the ruptured and scarred portions of the main pulmonary artery were stained with hematoxylin and eosin, 0.5% aqueous solution of toluidine blue, Verhoeff's elastic tissue method counterstained with Van Gieson,

Fig. 2.—Proximal portion of the pulmonary artery just above the valve ring, opened to show the recent rents. On the right can be seen a scarred area.

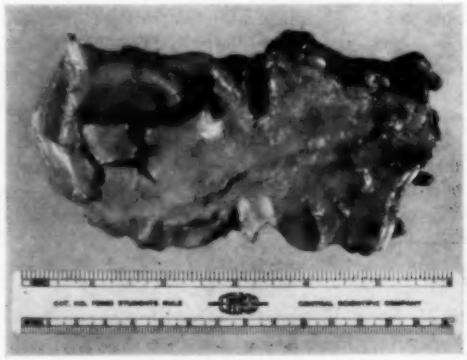


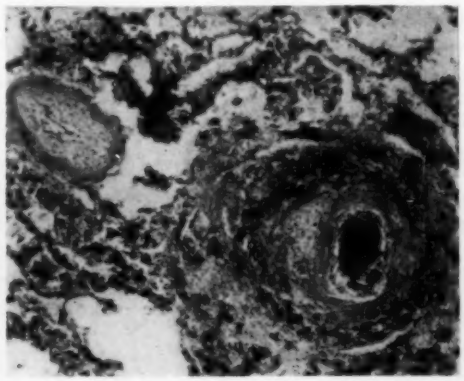
Fig. 3.—Section of the pulmonary artery through the edge of the recent tear. The wall is definitely thickened, and there are fragmentation and separation of the elastic fibers as compared with the normal in Figure 4. Elastic tissue stain; reduced about ¼ from mag. × 35.

Fig. 5.—Section of pulmonary artery from edge of scarred area. Note the intimal fibrosis and sharp interruption of the elastic fibers with fibrous tissue replacement. This was interpreted as a healed partial tear of the artery wall. Elastic tissue stain; reduced about ¼ from mag. × 35.

and the periodic acid-Schiff's reagent. Sections taken from the region of the rupture showed the tear extending through to the adventitia. In the five blocks examined, the wall of the artery was thickened owing to an excessive accumulation of metachromatic ground substance with an associated fragmentation and separation of the elastic fibers (Fig. 3). The degree of elastic tissue fragmentation far exceeded that seen in a normal pulmonary artery (compare Fig. 4). Only slight subendothelial fibrosis was seen in the region of the fresh tear. The adventitia showed a mild chronic inflammatory cell reaction with some sclerosis of the vasa vasorum. Sections from the area showing the old depressed scar revealed thick intimal fibrous tissue plaques with marked underlying medial damage. The only remaining elastic tissue was a few fragmented fibers adjacent to the adventitia. A rather striking feature was the sharp break in the media noted in the adjacent portion of the vessel (Fig. 5). Considerable collagenous fibrous tissue was present in the adventitia in this region. No lipid could be demonstrated in this area of intimal fibrosis. The aorta was carefully examined with the same stains, and no abnormality could be seen.

Fig. 4.—Normal pulmonary artery from a woman, 46 years old, for comparison with Figure 3. Note that the elastica is normally "fragmented" in the pulmonary artery as compared with the aorta, but in no way to the degree seen in Figure 3. Reduced about ¼ from mag. × 35.

Fig. 6.—Section of lung showing endoarteritis (upper left vessel) and necrotizing arteriolitis (lower right vessel). Hematoxylin and eosin stain; reduced about ¼ from mag. × 60.



Many of the medium and smaller arteries in the lung parenchyma showed medial hypertrophy and varying degrees of endarteritis. Rarely, an arteriole showed a definite necrotizing arteriolitis (Fig. 6). The lungs showed also the nodular hemosiderosis which has recently been described in cases of mitral stenosis.*

The mitral valve was thickened and distorted with dense collagenous fibrous tissue and was mildly vascularized. No active rheumatic nodules were seen in the valve cusp or in the adjacent auricular or ventricular muscle. However, the muscle did show small focal areas of perivascular fibrosis. The liver section showed the expected changes of marked chronic venous congestion with some cardiac cirrhosis. Sections of spleen, adrenals, and kidneys were unremarkable except for the effects of prolonged venous congestion. In the pancreas a chronic interstitial pancreatitis was present. There was a fine perilobular and periacinar fibrosis, with a mild mixed inflammatory cell infiltration.

The final pathological diagnoses were acute hemopericardium; dilatation and perforation of pulmonary artery; marked pulmonary arteriosclerosis; medial necrosis of pulmonary artery; cor pulmonale; mitral stenosis—rheumatic inactive; recent infarct of lung; cardiac cirrhosis of liver; generalized venous congestion.

COMMENT

Mitral stenosis is a frequent cause of pulmonary hypertension and, where the stenosis is severe, as in this case, the intrapulmonary pressure may reach excessive levels. In the 43 cases investigated by Yu and co-workers,[3] pulmonary artery mean pressures varied from normal to 86 mm. of mercury and sometimes approximated the mean systemic arterial pressure, even at rest. Although cardiac catheterization studies were not done in this case, the presence of pulmonary artery hypertension can be adduced from the accentuated pulmonic second sound, the Graham Steell murmur, and the markedly enlarged pulmonary artery. The enlargement of the artery was so marked as to have led to the mistaken clinical diagnosis of Lutembacher's syndrome, an error not uncommon in these circumstances.

The pain experienced by this woman during the last year of her life was characteristically anginal, being felt in the retrosternal area and coming only with exertion. Since there was no abnormality of the coronary arteries, it seems probable that the pain was due to distention of the pulmonary artery as suggested by Viar and Harrison.[4]

A review of the literature reveals 11 previously reported cases of rupture of the pulmonary artery. When Favorite [5] presented the ninth case, in 1934, he reviewed the eight cases which had been reported to that date.

In two of these the pathology is completely unknown, and in four other cases there was apparently aneurysmal dilatation of the pulmonary artery before rupture. In 1924 Moench [6] had described a case in which a widely patent ductus arteriosus had caused marked dilatation and subsequent rupture of the main pulmonary artery. Vogt-Møller [7] presented the case of a 4-year-old girl with congenital heart disease, in whom the arterial rupture was caused by a small streptococcal abscess in the vessel wall.

In Favorite's [5] case a patent ductus arteriosus associated with cor biatriatum triloculare produced the dilatation and rupture of the pulmonary artery. Another case was reported by Wilkinson,[8] in 1940, in which there was a marked congenital dilatation of the pulmonary artery and a bicuspid pulmonary valve. The only case of "spontaneous" rupture of the pulmonary artery was reported by Longland [9] in 1943. There was a 3-mm. complete tear in the wall of a grossly normal vessel which histologically showed some thinning of the media adjacent to the tear. This was presumed to be due to developmental hypoplasia.

The changes found in the smaller arteries and arterioles in the case presented were similar to those generally described as occurring in advanced mitral stenosis. In addition, the hyperplastic arteriosclerosis and

* References 1 and 2.

necrotizing arteriolitis, which was emphasized by Parker and Weiss,[10] were also seen. This condition of "pulmonary hypertension with malignant sclerosis" is seen in only the occasional advanced case of mitral stenosis.

The rupture of the main pulmonary artery appears to have been related to degeneration of medial elastic tissue. Brenner[11] showed that the elastic fibers of the pulmonary artery differ greatly from those in the aorta by being shorter, irregular, scanty, and more widely placed (Fig. 4). The definite degeneration of elastic fibers seen in this case, however, is evident when the vessel is compared with a normal specimen. The medial degeneration, consisting of fragmentation and loss of elastic fibers associated with increased amounts of metachromatic ground substance, is strikingly similar to the changes that occur in the aorta in medial necrosis. They have also been noted to a less degree in association with intimal fibrosis of the aorta, as recently described by Taylor,[12] who noted severe examples in patients with hypertension. It would thus seem reasonable to associate the medial degeneration with the prolonged and severe pulmonary hypertension resulting from the mitral stenosis.

Several writers † have described various changes which may be seen in the pulmonary artery as a result of rheumatic arteritis. However, in the sections of the pulmonary artery examined in this case, no definite evidence of old or recent inflammatory disease could be seen. The sharp break seen in the media in Figure 5 is of particular interest. The appearance would suggest that the patient had survived, sometime in the past, a tear involving the intima and most of the media. This observation also supports the concept that the fatal tear was on a degenerative rather than an inflammatory basis.

SUMMARY

The postmortem findings in a patient with advanced mitral stenosis are presented in which death was due to rupture of the pulmonary artery with resulting hemopericardium.

It is suggested that the rupture was due to degeneration of the elastic fibers in the pulmonary artery with an associated accumulation of metachromatic ground substance. This degeneration is comparable to medial necrosis of the aorta and was probably related to a severe pulmonary hypertension.

† References 10, 11, and 13.

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Studies of the Mechanism of Acute Vascular Reactions to Injury

I. The Relationship of Mast Cells and Histamine to the Production of Edema by Ovomucoid in Rats

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Redness, heat, and swelling are a constant part of the acute reaction to injury. These have been associated with the vascular component of the response. Histamine * and leukotaxin [2] have each been invoked as the chemical mediator of the response. Proof of the operation of either in vivo is circumstantial, and evidence that they are the only mediators is lacking.

Our earlier studies relating to this problem dealt with the edema produced by injection of testis extracts; † in more recent investigations ‡ we have centered our attention upon the hyperemia and edema produced in rats by the ovomucoid fraction of egg white. This phenomenon has, as we shall see, the basic characteristics of the acute small vessel reaction common to many injuries. It is easily and reproducibly demonstrated in a common laboratory animal and therefore amenable to a variety of observational techniques. We have, therefore, been examining this vascular injury on the premise that an accurate knowledge of its detailed mechanism would provide a basis for understanding the more complex injuries seen in inflammation.

The reaction produced by injection of egg white into rats was first described by Parker and Parker,[8] and it was redescribed by Selye.[9] The demonstration that ovomucoid is the active constituent of the egg white was made by Leger and Masson.[10] The pathogenesis of the phenomenon, that is, its cellular and chemical basis, is still unknown.

Graham and co-workers [11] associated histamine with the blood basophiles; Riley and West [12] showed the association of histamine with tissue basophiles (mast cells). Shachter and Talesnik [13] and Feldberg and Telesnik [14] showed release of histamine from rat tissues by egg white. This provided the basis for the following working hypothesis: The tissue histamine is associated with or resides in the mast cell; mast cells are associated with small blood vessels. Ovomucoid, and presumably other agents, cause the mast cell to release histamine, with the consequent known effects of histamine, namely, capillary hyperemia, increased permeability, and edema. The experiments which follow were designed to test this hypothesis.

MATERIAL AND METHODS

Animals

Female white rats (Sprague-Dawley) weighing 170 to 220 gm. were used. They were fed a standard rat diet (Rockland) and water ad lib. Group or individual cages were used as experiments warranted.

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* References 1 and 2.

† References 4 and 5.

‡ References 6 and 7.

Ovomucoid

This was prepared by the method of Hektoen and Cole.[15] Analyses of several preparations gave nitrogen (by micro-Kjeldahl method) 10.2%, hexosamine[16] 12.4%, and tyrosine, directly reacting with the Folin-Ciocalteau reagent, 3.0%.

Gross Observations

Observations were made of the external distribution of edema and of the degree of staining of tissues by intravenously administered Evans blue. On many animals observations were made of the distribution of internal bluing.

Dye Disappearance Measurements and Hemoglobin Concentration Estimations

The methods of measuring dye disappearance and hemoglobin concentration have been described previously.[17]

Histological Methods

Several methods of fixation were tried. Among these were 4% basic lead acetate, absolute alcohol, acetone, and neutral 4% formaldehyde. Freeze drying was also utilized for comparative purposes. The principle fixative used in most of the experiments described had the following composition: 80% alcohol, 4% formaldehyde (neutral), and 20% H_2O. In most instances tissues were imbedded in paraffin or the commercial preparation "Tissuemat." § Several staining procedures were employed; hematoxylin and eosin and toluidine blue preparations were made routinely. The latter stain was used as an 0.1% solution in 0.01 M acetic acid. Variations on this staining procedure with toluidine blue were used as indicated below. The periodic acid-Schiff stain was also used in some instances.

Enumeration of Mast Cells

Mast cells were counted in sections of the skin and in other organs. Tissue was removed from the circumoral region, the interscapular region of the back, and the dorsum of the four paws. The full thickness of skin down to the fascia was taken. This was fixed in alcohol-formalin, imbedded in paraffin, and sectioned at 5μ. The deparaffinized sections were stained in the acidified aqueous toluidine blue. The pH of this mixture was 3.9. The optical system used for counting was a 43 × achromatic objective and a pair of 12.5 × oculars. The diameter of the field with this combination was 305μ. Counts were made by four different observers, the majority being done by two of the four. The agreement among the observers was reasonable and for the most part within the limits of statistical expectation. Because of the obvious differences in numbers of cells in the corium and the subcutaneous areolar tissue (Fig. 1), we have adopted the procedure of counting equal numbers of fields encompassing the corium and the areolar tissue layers separately. In each tissue section, three fields were chosen at random from each layer. In some instances a small portion of the field belonging to the apposing layer was of necessity included. This happened more often in the areolar layer than in the corium.

Enumeration of Eosinophiles

Formalin- or alcohol-formalin-fixed paraffin sections of rat tissues have not, in our hands, proved suitable for the observation of these cells. Therefore, we resorted to making differential counts on subcutaneous tissue spreads from the feet and back regions of the animals. Spreads were prepared within a few minutes of death by gentle dissection of this layer from the overlying skin. They were air-dried, fixed five minutes in absolute methyl alcohol, and stained in Giemsa's mixture (National Analine Company) buffered at pH 5.0. This pH was chosen after a series of trials at pH's ranging from 4 to 6.5 showed it to give the maximum apparent number of eosinophiles relative to the number of mast cells. Differential counts were performed on the spreads in a manner similar to that used in

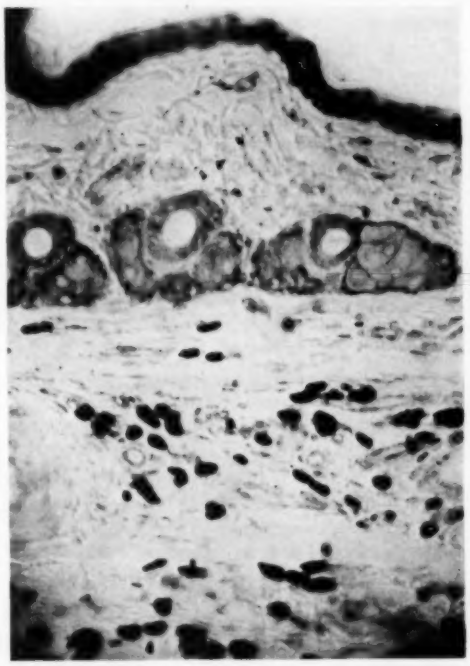


Fig. 1.—Skin and subcutaneous areolar tissue of dorsal skin of normal rat foot. Alcohol-formalin, 5μ section, toluidine blue, pH 3.9; reduced about ⅔ from mag. × 400.

§ Fischer Scientific Co., Pittsburgh.

counting blood smears. Each tissue spread was systemically scanned, the two cell types in each field being counted until a total of 200 cells had been accumulated.

Histamine Extraction

Histamine estimations were performed on tissue homogenates, prepared as follows: A sample (80 to 150 mg.) was weighed on a torsion balance and homogenized in 10 ml. of a modified Tyrode's solution.[18] The composition of this solution was as follows: NaCl, 8.5 gm.; KCl, 0.207 gm.; $MgCl_2$, 0.315 gm.; $CaCl_2$, 0.200 gm.; $NaHCO_3$, 1.00 gm.; glucose, 1.00 gm. per liter. Its pH as made ranged from 7.8 to 8.0. Homogenization was carried out in a glass homogenizer (Potter and Elvejhem) for a period of five to eight minutes, at room temperature and without cooling, until the tissue was uniformly dispersed and no gross tissue remained. It was centrifuged at approximately 1000 g for 10 minutes to sediment the gross particulate matter. The slightly opalescent supernatant fluid was decanted and stored frozen at —20 C until immediately prior to assay. We adopted this simple procedure on the basis of the following evidence. Originally, we used a modification of the procedure of Feldberg and Talesnik.[14] This consisted of homogenizing the tissue in 1.5 normal HCl, heating two minutes in boiling water, centrifuging to remove precipitate, and diluting and neutralizing to pH 7.4 with NaOH or $NaHCO_3$. The ease of release of histamine from rat skin seen with in vitro incubation suggested the possibility of using simple homogenization. We, therefore, compared this with heating in hydrochloric acid for two minutes and for one hour. The results of such an experiment on aliquots of a pool of dorsal feet skin is presented in Table 1. No significant difference is evident between the two methods. This method of extracting histamine, therefore, appears to be satisfactory for the rat skin. The average histamine content of samples of skin (whole feet) from 22 control animals was 64± 3γ/gm. of wet tissue. This agrees well with the value given by others.[14]

Table 1.—*Comparison of Methods of Extracting Histamine from Rat Feet Skin*

Method	Histamine, γ/Gm. Tissue
HCl + 100 C for 2 min	60, 67*
HCl + 100 C for 60 min	76, 70
Homogenization in Tyrode's solution	71, 61

* Each number represents the estimate on an individual sample.

Histamine Assay

This was performed on the terminal ileum of virgin female guinea pigs weighing between 215 and 450 gm. The bath volume was 16 ml. The modified Tyrode's solution used in the bath had the composition given above. Oxygen was bubbled through it for aeration and mixing. The assay procedure was similar to that of Code.[19] Atropine sulfate, 120γ per liter, was added to the Tyrode's solution. Assay values were computed in terms of histamine as free base. Replicate assays agreed within 10%, frequently within 5%.

Evidence for the specificity of the assay is the following: Material behaving as histamine is demonstrable in acid-acetone extracts of the skin of the several regions by paper chromatography with a butanol-acetic acid water solvent and identification with the modified Pauly reagent.[20] The material in the feet extracts is inhibited by an antihistamine (pyrilamine maleate [Neo-Antergan]) in the proportion described by Macintosh and Paton.[21] The active material is not potassium, since it takes at least 40 times the amount of potassium present in our most concentrated extracts to give a minimal response of the ileal strip. 5-Hydroxytryptamine, which is also present in skin extracts, does not contribute more than a few percent to the assayed histamine, for the following reason: We have not in several assays found more than 5γ to 6γ of 5-hydroxytryptamine per gram of skin. This is about one-fifth or less the amount of histamine present in the skin. The guinea-pig ileal strip requires about 10 times the quantity of 5-hydroxytryptamine, in our hands, as histamine to give an equivalent response. We have identified a third basic substance, having the mobility and color characteristics of tyramine,[20] in the chromatograms. This has no activity on the guinea pig ileal strip.

Tissue Water

This was estimated on 80 to 200 mg. aliquots of pooled skin from the four feet by drying to constant weight at 100 C for five days.

EXPERIMENTAL OBSERVATIONS

Gross Distribution and Character of the Edema Phenomena

The "egg white edema" phenomenon has been described in detail by others.‖ We repeat here certain significant features to be used in our later correlation. Two to three minutes after the intravenous injection of 3 mg. of ovomucoid hyperemia of the dorsal skin of the extremities is observed. About five minutes after injection the hyperemia pales somewhat, as the edema appears. The edema progresses to a maximum at about 30 minutes after the injection of ovomucoid.

‖ References 8-10 and 22.

Edema of the circumoral tissues becomes manifest in about the same time interval; hyperemia is not so clearly evident in this area. Injection of Evans blue (2 mg. per animal i. v.) reveals these areas more clearly. With the dye circulating at the time of ovomucoid injection, small focal areas of bluing are seen in the dorsal skin of the feet followed by the more diffuse bluing and edema. Additional areas also become evident when the dye is used to demonstrate the site of increased permeability. Thus, the base of the tail and the clitoris become blue. Coloration of the inner margins of the lips and under surface of the tongue may be evident. In addition, a very faint generalized bluing of the skin of the body in excess of that seen in dye-treated control animals can also be made out. Internally, the only region showing any significant bluing is the thymus. Bluing of the lining of the trachea is seen about as often in control animals as in those injected with ovomucoid. The regional lymphatics and lymph nodes of the extremities frequently show the blue dye by 30 minutes after the ovomucoid injection.

The skin of the affected regions when removed shows a gelatinous edema of the subcutaneous areolar tissue. Histologically the affected areas show dilated small vessels, capillaries, and venules, packed with erythrocytes, and an edema confined mainly, if not entirely, to the subcutaneous vascular areolar tissue layer. The edema fluid contains protein which stains with eosin and by the periodic acid-Schiff method. Lymphatics can be seen to be dilated. Extraction of the edematous skin with saline and comparison with extracts of normal skin by electrophoresis show the constituent proteins to be those of the plasma.

The physiological correlates of the phenomena have been described by Halpern and Briot.[22] As a part of our observations, we have routinely measured the rate of disappearance of Evans blue from the circulation. This we consider to be an index of plasma protein loss. Also we have observed the changes in hemoglobin concentration as an index of plasma volume change. Administration of a given dose of ovomucoid causes an acceleration of the disappearance of Evans blue from the circulation. The rate of dye disappearance is correlated with the degree of edema of the exposed parts, at doses up to 3 mg. of ovomucoid per animal. With higher doses, there is a small further increase in rate of dye disappearance, without visible or measurable increase in edema of the feet but associated with more evident plasma leakage in the other parts of the skin and around the thymus. The reduction in plasma volume, as measured by rise in hemoglobin concentration, roughly parallels the leakage of plasma protein demonstrated by dye loss.

TABLE 2.—*Mast-Cell and Histamine Content of Rat Skin and Subcutaneous Tissues*

Site	Mast Cells, No./HPF	Histamine, γ/Gm.
Back		
Upper layer	3.6 ± 0.4*	16 ± 4
Whole skin	3.3 ± 0.3	10 ± 1
Lower layer (s. c.)	3.1 ± 0.4	28 ± 3
Snout		
Upper layer	7.1 ± 0.6	45 ± 8
Whole skin	8.8 ± 0.4	45 ± 13
Lower layer (s. c.)	10.4 ± 0.8	52 ± 11
Extremities		
Upper layer	7.3 ± 0.3	33 ± 3
Whole skin	12.2 ± 0.3	64 ± 3
Lower layer	17.1 ± 0.5	119 ± 15

* All data given as the mean and standard error.

DISTRIBUTION OF MAST CELLS

Mast cells are not easily seen in hematoxylin- and eosin-stained preparations; with toluidine blue following alcohol-formalin, freeze drying, or even neutral formalin fixation, the numerous mast cells of the rat become evident. Table 2 gives the distribution of mast cells in the corium and areolar tissues of the skin from six normal rats. The high population in the feet and snout skin as contrasted to the dorsal body skin is clearly evident. It is further evident that the subcutaneous areolar tissue of the dorsal feet skin has the highest population. We have also performed mast-cell counts on the viscera of rats. Such counts on visceral tissue suffer from the fact that the mast cells are focally distributed, being situated in the perivascular

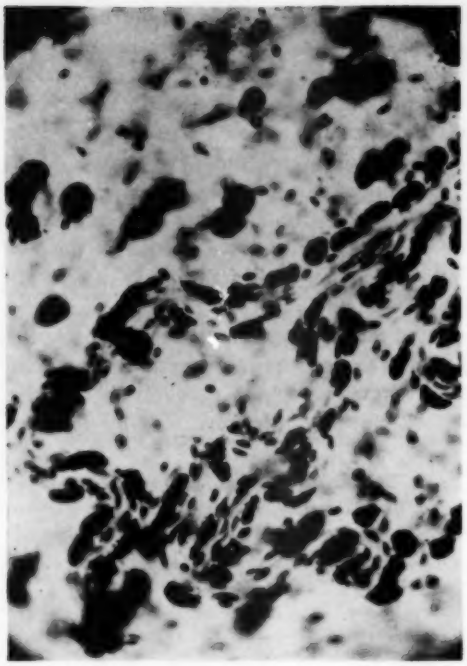


Fig. 2.—Subcutaneous areolar tissue of dorsal skin of normal rat foot. Incubated in Tyrode's solution 10 minutes. Tissue spread stained in 50% alcohol, 0.1% toluidine blue, 0.01 M acetic acid; reduced about ⅔ from mag. × 400.

and subserosal areolar tissue. Furthermore, these cells are frequently difficult to distinguish among certain highly basophilic glandular elements. It is of interest, nevertheless, that of all of the viscera so examined the thymus appears to have the highest number.

The close association of the mast cells with the small blood vessels is another characteristic of their distribution.[12] This is most striking in the subcutaneous tissue of the feet, as shown in Figure 2. In the back this is less pronounced, and in the mesentery it appears less frequently than the isolated groups of cells distant from the visible vessels.

Correlation Between Mast-Cell Numbers and Histamine Content of the Several Tissues

Extracts of whole skin and extracts of the separated deep areolar and superficial compact layer of the skin were assayed for histamine. The results are shown in Table 2. A plot of these values reveals a very good linear correlation, as shown in Chart 1. Also evident is the fact that extrapolation of the line to zero mast-cell count coincides with zero histamine content of the tissue. Thus, histamine, in the skin and subcutaneous tissues of the rat, is closely associated with the presence of this cell type.

In assays of visceral tissue for histamine, performed in the same manner as the cutaneous tissue assays, the thymus and the glandular portion of stomach yield the highest activity, 25γ and 48γ histamine per gram of wet tissue, respectively.

Can the Eosinophiles Account for the Histamine Content of the Tissues Under Consideration?

Because of the suggested association of the eosinophiles of the blood with histamine,[19] we examined the tissue content of these cells in two areas having widely different mast-cell populations and correlated histamine concentration—the areolar tissue of the back and the areolar tissue of the feet. The ratio of eosinophiles to mast cells was found to be 2 : 3 for the back and 1 : 200 for the feet. These represent the averages of two samples of each tissue from each of five

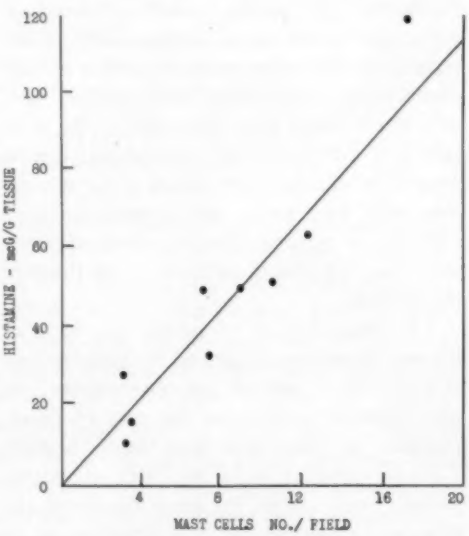


Chart 1.—The relationship of histamine to mast-cell content of rat tissues.

animals. From the estimates of number of mast cells per HPF one calculates that there are about 2 eosinophiles per HPF in the back tissues and 0.1 eosinophiles per HPF in the feet tissues. Thus, there is no positive correlation between tissue eosinophiles and histamine in these locations.

The in Vitro Release of Histamine by Ovomucoid from the Isolated Feet Skin of Rats and the Associated Disruption of Mast Cells

Many substances are now known which will release histamine from tissues.¶ Several of these substances have also been shown to cause changes in mast cells.# The following experiments were performed to see if ovomucoid applied directly to the tissue in vitro would effect mast-cell changes and histamine release.

Table 3.—*In Vitro Release of Histamine from Rat Feet Skin by Ovomucoid*

Test Substance	Concentration of Test Substance in Tyrode's Solution, Mg./Ml.	Histamine Free in Medium After 10 Min. Incubation, γ/Gm. of Tissue
None	0	7, 7
		3, 5
Ovomucoid	0.05	2
	0.5	25, 19
	1.5	17, 14
	2.5	21
	4.5	16, 17
	5.0	22
Bovine serum albumin	4.5	4, 7
Bovine serum gamma globulin	4.5	5, 8

The dorsal skin and subcutaneous tissue of the feet were removed gently and incubated in the modified Tyrode's solution used for the guinea pig ileal strip. The temperature of incubation was 25 to 28 C, and oxygen was bubbled through the solution. Preliminary trials showed that the major portion of histamine appearing in the incubating medium with or without added releaser appears within the first 10 minutes. Therefore, most of the experiments measuring the effects of ovomucoid and other substances were performed using this time interval.

Table 3 shows the composite results of several such experiments. It is evident that ovomucoid is capable of releasing histamine in excess of the spontaneous release from rat feet skin; the release by ovomucoid appears to be a relatively specific phenomenon, since it is not given by several proteins. It is interesting that ovomucoid appears to show a threshold. That is, in concentrations under 0.5 mg. per milliliter of incubating medium there is no release in excess of the control. At and beyond this concentration the maximal release appears to occur, and a 10-fold increase does not elicit more histamine from the tissue. The maximum quantity of histamine released is only about one-third of the total released from the tissues by homogenization. This suggests that there is a limiting factor in the system, which may be either the simple physical effect of diffusion of ovomucoid into the tissues and histamine from it or some unknown factor.

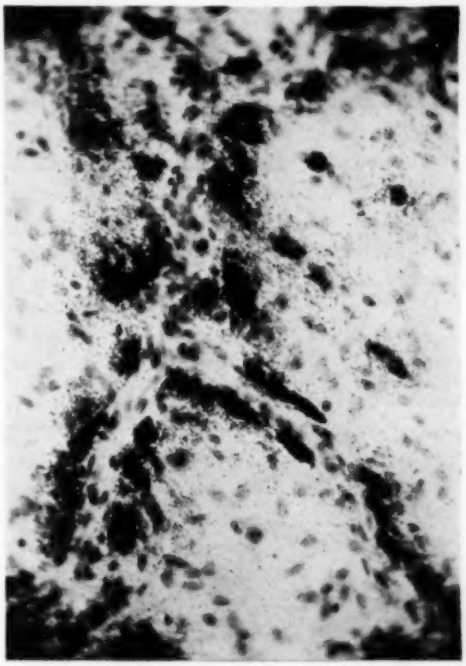


Fig. 3.—Subcutaneous areolar tissue of dorsal skin of normal rat foot. Incubated 10 minutes in Tyrode's solution containing 2.5 mg. ovomucoid per milliliter. Prepared and stained in same manner as control in Figure 2; reduced about ⅖ from mag. × 400.

¶ References 13, 21, 23, and 24.

References 24-26.

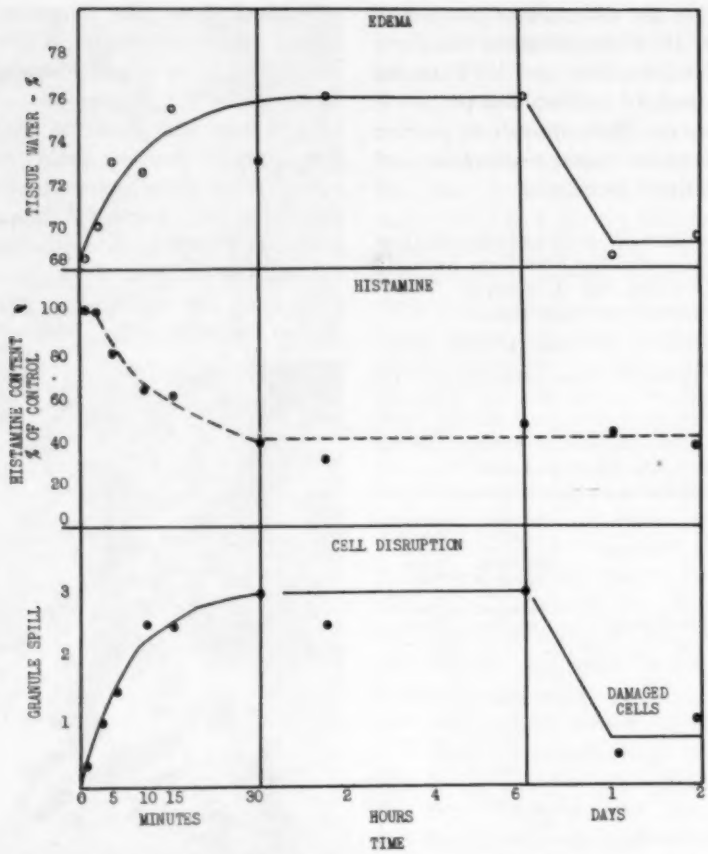


Chart 2.—The relationship in time between mast-cell disruption, histamine release, and edema formation.

Examination of the tissues following such in vitro treatment with ovomucoid reveals many "exploded" mast cells, as seen in Figure 3. This apparent explosion of cells is characterized by a relatively uniform dispersion of spilled granules radially distributed around each cell. The control tissue incubated in Tyrode's solution is shown in Figure 2. The difference between the two is quite apparent. Not all of the cells are "exploded" in a tissue treated with a concentration of ovomucoid causing release of histamine; some disrupted cells are frequently visible in tissues incubated in the control medium as well as in unincubated fresh spreads. In the latter type of disruption, the granules are nonuniformly dispersed and this is correlated with mechanical handling apparent along the lines of cleavage and tearing and in areas which obviously have been manipulated. With some care preparations can be made in which the changes attributable to the ovomucoid and other releasing agents are clearly demonstrable.

Release of Histamine in Vivo Following a Single Dose of Ovomucoid and the Associated Tissue and Mast-Cell Changes

The following experiments were designed to observe the relationship in time between the development of edema, the alterations in mast cells, and the tissue histamine content.

The dose of ovomucoid used in these experiments was 15 mg. per animal. This dose was chosen after preliminary experiments had shown it to cause clearly evident mast-cell disruption. Observations were made on treated and saline-injected control

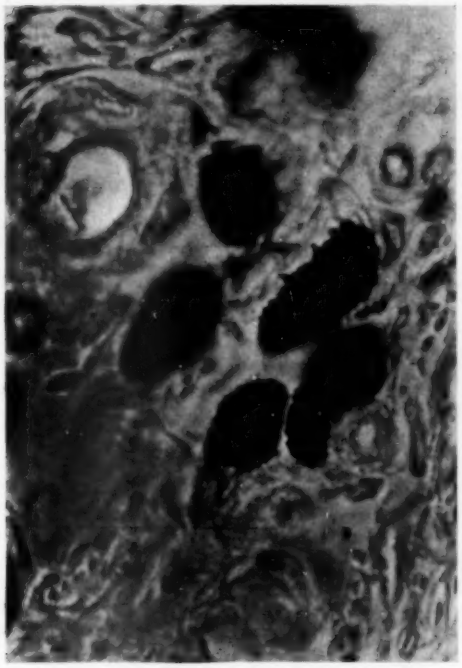
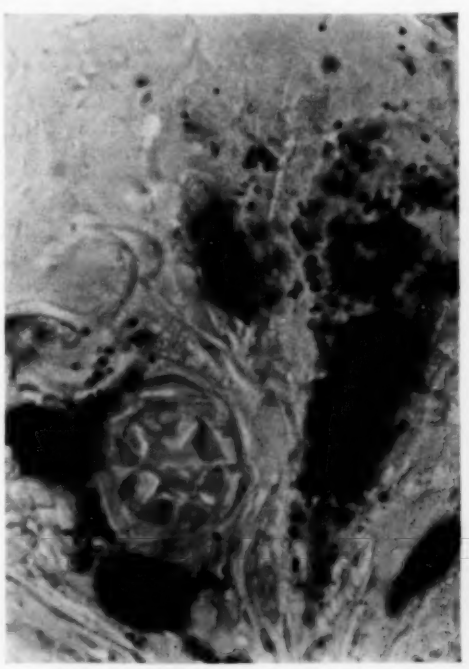


Fig. 4.—Mast cells from subcutaneous areolar tissue of rat foot. Control. Alcohol-formalin, toluidine blue stain, 5μ; reduced about ⅔ from mag. × 2425.

animals at intervals through a total period of 48 hours following administration of the ovomucoid. The data are shown in Chart 2. The values for each point represents averages of two, three, or four and, in the case of the controls, nine observations on individual animals. The measure of the edema response is given in terms of the increase in percentage of tissue water. The stability of the normal tissue water (68.4 with a standard deviation of ±1.21% for nine control determinations) makes small rises of significance. Histamine was calculated as micrograms of free base per gram of dry tissue. The comparison on a dry weight basis was made because of the large fluctuations in wet weight accompanying the edema. The wet weight of the tissue may increase by nearly 100% with the maximal degree of edema. For representation of the experimentally induced changes, the values for histamine were recomputed as percent of the tissue histamine of the control animals. The mast cells were examined in toluidine-blue-stained tissue sections, and the relative numbers of disrupted cells were recorded as 0 to 3+, the latter being the situation in which practically all cells showed some degree of disruption.

The relationship of the changes in time is shown in Chart 2. Edema rises to a maximum in 30 to 60 minutes. It remains maximal through the 6th hour and by the 24th hour disappears. A portion of the histamine disappears from the tissues simultaneously with the appearance of edema and declines to a minimum of about 40% in this series at 30 minutes. It remains at this low level through the next 48 hours and probably longer. Evidence of mast-cell disruption and granule-spill, in excess of the mechanical disruption from removal of tissues, becomes apparent within the first few minutes (Fig. 5) and increases to a maximum parallel to tissue edema and histamine disappearance. Spilled granules in large numbers are evident through the 6th hour after initiation of the vascular injury; by the 24th hour there are far fewer granules evident in the tissues. By actual count there is little or no decrease in the numbers of identifiable mast cells, but a large proportion of the cells have the appearance shown in Figure 6, as contrasted with

Fig. 5.—Mast cells from subcutaneous areolar tissue of rat foot. Animal received 15 mg. ovomucoid i. v. 10 minutes prior to killing. Notice "explosive" spill of granules. Tissue prepared as in Figure 4; reduced about ⅔ from mag. × 2425.



the normal configuration, seen in Figure 4. These damaged cells are partly or largely degranulated and smaller than the normal cells; their nuclei are more clearly visible.

Relationship Between Histamine Content of the Tissue and Its Capacity to Respond with Edema Formation Following Repeated Injection of Ovomucoid

Having seen the disruption of mast cells in vitro and in vivo and the parallel release of histamine with the associated edema production, we next sought to determine what relationship might exist between the degree of edema response and the residual histamine level. Preliminary experiments had shown that with repeated daily intravenous injections of 10 mg. of ovomucoid per animal the gross edema phenomenon did not appear after the second or third injection. The quantitative experiment was designed as follows:

Animals in groups of six were each given daily intravenous injection of 10 mg. of ovomucoid. Three animals from each group were used for estimating tissue histamine, and three were challenged with 3 mg. of ovomucoid for measurement of edema response. Tissue histamine was also measured in these animals. The 3 mg. dose of ovomucoid will, we have found, produce just maximal skin edema, and we have used it in many experiments as the challenging dose. Thus, in the total experiment the histamine concentration and edema response were measured in previously untreated animals, in animals 24 hours after a single treatment, after two doses of ovomucoid (48 hr.), and after three doses of 10 mg. of ovomucoid (72 hr.) each. The data are plotted in Chart 3. Edema response is here indicated by the increase in percentage of tissue water, and histamine is indicated as before.

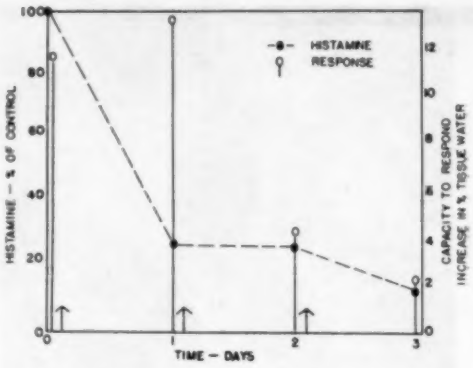
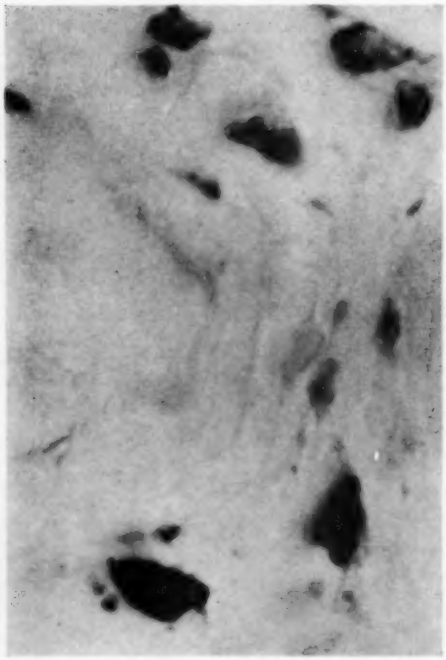


Chart 3.—Correlation between histamine content of skin and capacity to respond with edema after repeated injections of ovomucoid.

Fig. 6.—Mast cells showing various degrees of degranulation and damage 24 hours after second dose of 10 mg. of ovomucoid i. v. Same type of preparation as in Figure 4; reduced about 2/5 from mag. × 2425.



It is clearly apparent that the tissue histamine drops almost to the minimum value after the first injection of ovomucoid. Only a small further drop is seen after the third injection, but the response with edema is not reduced after the first injection despite the low concentration of tissue histamine. Edema response is reduced after the second and third injections. These observations have been confirmed on other trials. Nearly the maximum change apparent in the tissue mast cells is seen by 24 hours after the first injection of 10 mg. of ovomucoid. These changes consist of degranulation of the cells to a varying degree, with reduction in apparent size of the cells (Fig. 6). The severity of the changes seems slightly greater at 48 hours (i. e., after the second administration of 10 mg. of ovomucoid) than at 24 hours, but the difference is not great.

COMMENT

The data presented confirm the claims of Riley and West [12] that the histamine content of a tissue is related to its mast-cell content. Within the sphere of our observations there is a linear correlation between mast-cell number and histamine content of the tissues. There is no apparent relationship of tissue eosinophiles to histamine content. The in vitro release of histamine and the associated disruption of mast cells by ovomucoid is shown. Ovomucoid in vivo is shown to release histamine and "explode" mast cells. A correlation between the distribution of the edema evoked by intravenous injection of ovomucoid and the distribution of mast cells is evident. These facts taken together with the temporal correlation in vivo of the mast-cell alterations, histamine release, and appearance of edema lend support to the hypothesis that ovomucoid in causing mast-cell disruption releases histamine and that histamine, presumably, causes the vascular hyperemia and increased permeability.

TABLE 4.—*Minimum Quantity of Histamine Lost from the Tissue with Production of Gross Edema*

Treatment	Skin Histamine Content, γ/Gm. Dry Wt.	Tissue Water Content, %
Saline 30 min. prior to killing.....	259	69.4
	176	68.2
	203	67.8
	211	69.3
	281	68.5
Average.....	226	68.6
3 mg. ovomucoid 30 min. prior to killing	250	79.8
	182	77.6
	292	79.2
	183	79.1
	118	82.2
Average.....	205	78.2

The discrepancy between the histamine levels of the tissue and manifest tissue edema following multiple injections of ovomucoid demands explanation. Several hypotheses are possible; the simplest of these is that the release of only a small portion of the histamine contained in the cells is necessary to produce the vascular changes. There is evidence that this is so: We have observed in several experiments that the injection of a dose of ovomucoid (3 mg. per animal) which will produce pronounced edema of the extremities may be associated with little or no apparent decrease in the tissue histamine content (Table 4). Intracutaneous injections of histamine in the back and subcutaneous injections in the feet show that the minimum concentration necessary to cause leakage of intravascular Evans blue into the local site ranges between 10γ and 100γ/ml. of injected fluid. We have estimated the concentration of histamine in mast cells, assuming it to be there, to be about 10,000γ/ml. of cells.* If 1% of this were spilled into a volume equal to twice the volume of the mast cell then the concentration in this volume would be 50γ/ml., a concentration apparently sufficient to produce the vascular changes. The close approximation of the mast cells to the small vessels (Fig. 2) makes this a reasonable possibility. This formulation may explain the fact that a full edema response occurs with a residual apparent tissue histamine of less than 30% of normal. It does not explain why the edema does not appear after two or more large doses of ovomucoid and in the presence of about the same quantity of residual histamine. We must, therefore, consider other possibilities; at the moment several seem reasonable. One is that the cells, because of differing position in relation to the vessels,[27] are not equally susceptible to active material circulating in the blood. Or that, if equally susceptible, the histamine released from a cell more distant from a vessel wall may reach the responsive site in a concentration too low to be effective. Another possibility is that there is a mechanism for releasing histamine from the cell or tissue site and that this releasing mechanism is damaged by repeated injections of ovomucoid.

Finally, the possibility must be considered that there are substances other than histamine in the tissue involved in the reaction and not under scrutiny. One substance, 5-hydroxytryptamine (serotonin), is present

* Benditt, E. P.: To be published.

in the tissue and is capable, when injected into the subcutaneous tissue of the feet, of producing an intense local edema.† What role this may play in the reaction remains to be ascertained.

Our attention has been confined primarily to ovomucoid as an incitant of the local vascular response. Other chemical moieties of biological origin have been demonstrated to produce a similar response in animals.[28] It seems likely that we shall find, on further exploration, substances derived from bacteria or from damaged tissue cells which like ovomucoid, Compound 48/80 (a condensation product of *p*-methoxyphenylethyl-methylamine and formaldehyde), and dextran are capable of setting off this common phenomenon of hyperemia and increased small vessel permeability.

The evidence, ours and that of other investigators, begins to elucidate the structural-functional system involved in the acute response of small vessels to injury. The mast cell seems to be an important entity in this reaction. Its full role and the role of other cells and substances remain to be defined.

SUMMARY AND CONCLUSIONS

Egg white edema, produced by intravenous injection in rats of the ovomucoid fraction of egg white, has been used as a model reaction for small vessel injury. Observations of the gross phenomenon, its distribution, and certain physiological correlates were made. Cellular and chemical changes in the tissue associated with the appearance of the edema phenomenon were observed. The following conclusions are drawn: 1. The gross distribution of the sites in which the hyperemia and increased vascular permeability occur are correlated with the density of mast cells in the tissue. 2. The histamine content of the skin and subcutaneous tissue is related to its mast-cell concentration. 3. Ovomucoid "explodes" mast cells and releases histamine in vitro and in vivo. 4. A temporal correlation exists between mast-cell disruption, histamine release, and edema formation in the most reactive area, the dorsal feet skin, following injection of a single large dose of ovomucoid. 5. A large reduction in histamine content of the tissue is observed following a single large dose of ovomucoid. Nevertheless, vascular injury manifested by edema develops to the maximal degree when the injurious agent is reapplied. After multiple injections of the injuring substance edema is almost nil despite little further drop in histamine content. This raises the question of whether histamine is the sole or perhaps even the main mediator of this injury response. Other possible facets of the mechanism are discussed.

Miss Margaret Arase and Miss Constance Corley assisted in this work.

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Books

Surgery of Pulmonary Tuberculosis. By James H. Forsee, A.B., B.S., M.D., F.A.C.S., F.A.C.P.; Col. (MC), U. S. Army; Chief, Surgical Services, Fitzsimons Army Hospital, Denver. Price, $6.50. Pp. 208, with 59 illustrations (1 in color), 11 graphs, 46 tables. Lea & Febiger, 600 S. Washington Sq., Philadelphia 6, 1954.

This valuable monograph by Colonel Forsee has been a much needed volume. There has been no recent book written exclusively on this subject which reflects American experience in the surgical treatment of pulmonary tuberculosis. The past eight years have produced a great revolution in the treatment of this disease. Streptomycin and other antituberculous drugs have expedited the medical management and have enhanced the wider application of surgical therapy.

This monograph has been divided into three appropriate sections: (1) Principles in the Application of Surgery in Pulmonary Tuberculosis; (2) Operative Surgical Procedures, and (3) Experience with Surgical Therapy in Pulmonary Tuberculosis. Each section is concisely and authoritatively written. Throughout his monograph the author clearly presents the great advances in phthisiology of the past eight years brought about by the advent of chemotherapy of pulmonary tuberculosis. From a vast source of clinical material over many years, he has compared the long-range studies of collapse therapy to those of more recent extirpative surgery. The indications and type of resection employed, as well as the chemotherapeutic regimen and length of hospitalization, are presented in considerable detail. The importance and advantages of a "tuberculosis therapy team" are made obvious, and the plea for better utilization of general hospital facilities in the care of tuberculosis patients is emphasized.

For the pathologist and bacteriologist this dissertation will serve as a useful guide in classifying types and phases of the tuberculous process in relation to the indications and timing of resective surgery. The radiologist and pathologist will be pleased by the clinical correlation of routine and special x-ray procedures and the pathological findings. For those that treat and diagnose tuberculosis, be they surgeon, internist, radiologist, pathologist, or bacteriologist, this monograph will be most useful.

Fat Metabolism (A Symposium on the Clinical and Biochemical Aspects of Fat Utilization in Health and Disease). Edited by Victor A. Najjar. Price, $4.50. Pp. 185. The Johns Hopkins Press, Homewood, Baltimore 18, 1954.

This little book is the outcome of a symposium sponsored by the McKesson & Robbins Laboratories, of Columbus, Ohio. Participants in the conference discussed various clinical and biochemical features of fat metabolism. Among these are discussions on obesity in childhood, multiple causative factors in obesity, endocrine factors in obesity, lipemia, essential hyperlipemia, the use of fat emulsions in intravenous alimentation, lipemia as a clearing factor in lipid transport, the role of coenzyme A in fat metabolism, enzymatic oxidation and synthesis of fatty acids, and a number of other topics. Among the participants were Fritz Lipmann, Albert Lehninger, Christian Anfinsen, Jean Mayer, L. Emmett Holt Jr., Victor Najjar, and others. Each discussion carries with it a bibliography dealing with recent literature, and there are numerous charts, photographs, and tables. The material is also indexed. This book will be of value to those who are interested in the more recent developments relating to fat metabolism.

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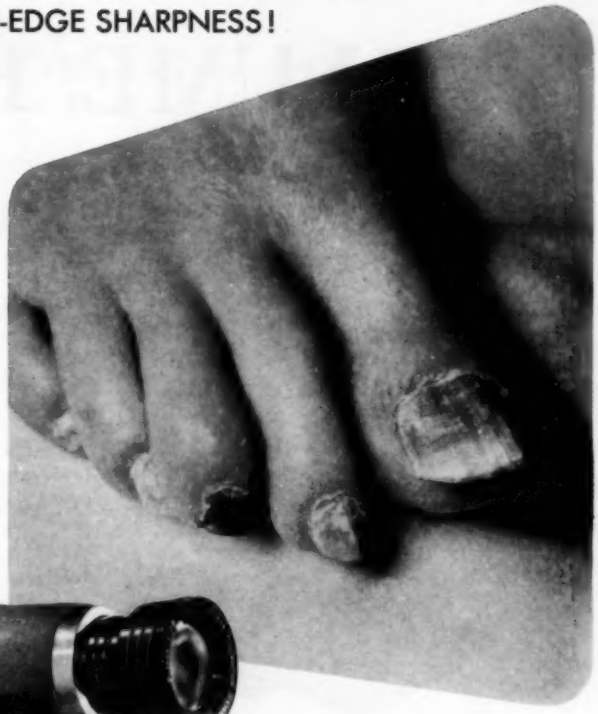

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Often points to presence of subclinical inflammatory reactions[2]

"The detection of CRP in the serum therefore serves as an indicator of the intensity of an inflammatory process . . . the most consistently positive laboratory test in the presence of rheumatic activity."

C·R·P·A

Close correlation between presence of C-reactive protein and rheumatic activity[3]

"Absence of this protein during the diagnostic examination would exclude active rheumatic fever even though other values, such as the erythrocyte sedimentation rate, were abnormal."

C·R·P·A

Level of C-reactive protein a measure of inflammation in acute myocardial infarction[4]

"a positive test for CRP is a non-specific but sensitive indicator of inflammation of infectious or non-infectious origin . . . useful in evaluating the subsidence of the inflammatory process in the heart."

C·R·P·A

An aid in deciding when inflammation has subsided[5]

"an aid in deciding when inflammation has subsided . . . useful in following the course of rheumatic fever in the same sense that the erythrocyte sedimentation test is. Like the latter test it is nonspecific and can be caused by any infection . . . but is more easily interpreted . . ."

1. Wood, H. F., and McCarty, M.: Am. J. Med. *17*:768 (Dec.) 1954. 2. Stollerman, G. H., et al.: Am. J. Med. *15*:645 (Nov.) 1953. 3. Rantz, L. A.: Disease-a-Month *1*:18 (Oct.) 1954. 4. Kroop, I. G., and Shackman, N. H.: Proc. Soc. Exper. Biol. & Med. *86*:95 (May) 1954. 5. McEwen, C.: M. Clin. North America (Mar.) 1955, to be published.

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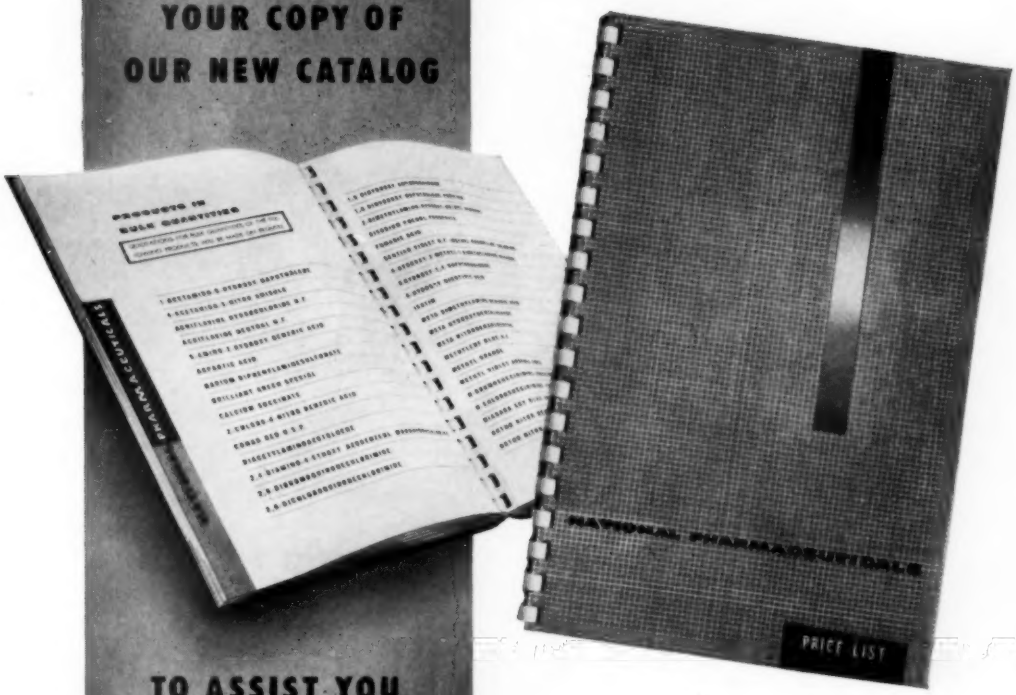

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